THE SCIENCE OF BEVERAGES
VOLUME 5

FERMENTED BEVERAGES

Edited by
Alexandru Mihai Grumezescu
Alina Maria Holban

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ALEXANDRU MIHAI GRUMEZESCU

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SERIES PREFACE

Food and beverage industry accounts among the most developed sectors, being constantly changing. Even though a basic beverage industry could be found in every area of the globe, particular aspects in beverage production, processing, and consumption are identified in some geographic zones. An impressive progress has recently been observed in both traditional and modern beverage industries and these advances are leading beverages to a new era. Along with the cutting-edge technologies, developed to bring innovation and improve beverage industry, some other human-related changes also have a great impact on the development of such products. Emerging diseases with a high prevalence in the present, as well as a completely different lifestyle of the population in recent years have led to particular needs and preferences in terms of food and beverages. Advances in the production and processing of beverages have allowed for the development of personalized products to serve for a better health of overall population or for a particular class of individuals. Also, recent advances in the management of beverages offer the possibility to decrease any side effects associated with such an important industry, such as decreased pollution rates and improved recycling of all materials involved in beverage design and processing, while providing better quality products.

Beverages engineering has emerged in such way that we are now able to obtain specifically designed content beverages, such as nutritive products for children, decreased sugar content juices, energy drinks, and beverages with additionally added health-promoting factors. However, with the immense development of beverage processing technologies and because of their wide versatility, numerous products with questionable quality and unknown health impact have been also produced. Such products, despite their damaging health effect, gained a great success in particular population groups (i.e., children) because of some attractive properties, such as taste, smell, and color.

Nonetheless, engineering offered the possibility to obtain not only the innovative beverages but also packaging materials and contamination sensors useful in food and beverages quality and security sectors. Smart materials able to detect contamination or temperature differences which could impact food quality and even pose a hazardous situation for the consumer were recently developed and some are already utilized in packaging and food preservation.

This 20-volume series has emerged from the need to reveal the current situation in beverage industry and to highlight the progress of the last years, bringing together most recent technological innovations while discussing present and future trends. The series aims to increase awareness of the great variety of new tools developed for traditional and modern beverage products and also to discuss their potential health effects.

All volumes are clearly illustrated and contain chapters contributed by highly reputed authors, working in the field of beverage science, engineering, or biotechnology. Manuscripts are designed to provide necessary basic information in order to understand specific processes and novel technologies presented within the thematic volumes.

Volume 1, entitled *Production and management of beverages*, offers a recent perspective regarding the production of main types of alcoholic and nonalcoholic beverages. Current management approaches in traditional and industrial beverages are also dissected within this volume.

In Volume 2, *Processing and sustainability of beverages*, novel information regarding the processing technologies and perspectives for a sustainable beverage industry are given.

Third volume, entitled *Engineering tools in beverage industry* dissects the newest advances made in beverage engineering, highlighting cutting-edge tools and recently developed processes to obtain modern and improved beverages.

Volume 4 presents updated information regarding *Bottled and packaged waters*. In this volume are discussed some wide interest problems, such as drinking water processing and security, contaminants, pollution and quality control of bottled waters, and advances made to obtain innovative water packaging.

Volume 5, *Fermented beverages*, deals with the description of traditional and recent technologies utilized in the industry of fermented beverages, highlighting the high impact of such products on consumer health. Because of their great beneficial effects, fermented products still represent an important industrial and research domain.

Volume 6 discusses recent progress in the industry of *Nonalcoholic beverages*. Teas and functional nonalcoholic beverages, as well as their impact on current beverage industry and traditional medicine are discussed.

In Volume 7, entitled *Alcoholic beverages*, recent tools and technologies in the manufacturing of alcoholic drinks are presented. Updated information is given about traditional and industrial spirits production and examples of current technologies in wine and beer industry are dissected.

Volume 8 deals with recent progress made in the field of *Caffeinated and cocoa-based beverages*. This volume presents the great variety of such popular products and offers new information regarding recent technologies, safety, and quality aspects as well as their impact on health. Also, recent data regarding the molecular technologies and genetic aspects in coffee useful for the development of high-quality raw materials could be found here.

In Volume 9, entitled *Milk-based beverages*, current status, developments, and consumers trends in milk-related products are discussed. Milk-based products represent an important industry and tools are constantly been developed to fit the versatile preferences of consumers and also nutritional and medical needs.

Volume 10, *Sports and energy drinks*, deals with the recent advances and health impact of sports and energy beverages, which became a flourishing industry in the recent years.

In Volume 11, main novelties in the field of *Functional and medicinal beverages*, as well as perspective of their use for future personalized medicine are given.

Volume 12 gives an updated overview regarding *Nutrients in beverages*. Types, production, intake, and health impact of nutrients in various beverage formulations are dissected through this volume.

In Volume 13, advances in the field of *Natural beverages* are provided, along with their great variety, impact on consumer health, and current and future beverage industry developments.

Volume 14, *Value-added Ingredients and enrichments of beverages*, talks about a relatively recently developed field which is currently widely investigated, namely the food and beverage enrichments. Novel technologies of extraction and production of enrichments, their variety, as well as their impact on product quality and consumers effects are dissected here.

Volume 15, *Preservatives and preservation approaches in beverages,* offers a wide perspective regarding conventional and innovative preservation methods in beverages, as well as main preservatives developed in recent years.

In Volume 16, *Trends in beverage packaging*, the most recent advances in the design of beverage packaging and novel materials designed to promote the content quality and freshness are presented.

Volume 17 is entitled *Quality control in the beverage industry*. In this volume are discussed the newest tools and approaches in quality monitoring and product development in order to obtain advanced beverages.

Volume 18, *Safety issues in beverage production*, presents general aspects in safety control of beverages. Here, the readers can find not only the updated information regarding contaminants and risk factors in beverage production, but also novel tools for accurate detection and control.

Volume 19, *Biotechnological progress and beverage consumption*, reveals novel tools used for advanced biotechnology in beverage industry production.

Finally, Volume 20 entitled *Nanoengineering in the beverage industry* take the readers into the nanotechnology world, while highlighting important progress made in the field of nanosized materials science aiming to obtain tools for a future beverage industry.

This 20-volume series is intended especially for researchers in the field of food and beverages, and also biotechnologists, industrial representatives interested in innovation, academic staff and students in food science, engineering, biology, and chemistry-related fields, pharmacology and medicine, and is a useful and updated resource for any reader interested to find the basics and recent innovations in the most investigated fields in beverage engineering.

Alexandru Mihai Grumezescu
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PREFACE

Fermented foods and beverages are widely produced and consumed. This industry has impacted greatly the science of beverages, since fermentation not only provides additional nutrients, special taste, and flavor to the drinks, but is also a much healthier alternative to many products. Since ancient times, fermented beverages have been developed constantly and their production, processing, and preservation approaches were significantly updated during time. However, some highly appreciated fermented products are still produced by respecting traditional and original procedures. The purpose of this book is to review and discuss new trends and applications of fermented beverages, recent tools used for their processing and characterization, and also innovative biotechnological protocols to develop new and healthier drinks.

This volume contains 15 chapters prepared by outstanding authors from India, Spain, Germany, Romania, Croatia, Turkey, Jordan, Niger, South Africa, Bulgaria, Brazil, and México.

The selected manuscripts are clearly illustrated and contain accessible information for a wide audience, especially food and beverage scientists, engineers, biotechnologists, biochemists, industrial companies, students and also any reader interested in learning about the most interesting and recent advances in the field of beverage science.

Chapter 1, *Fermented beverages and their health benefits,* by Mitali Makwana et al., introduces to the readers the wide variety of fermented products, their nutritional and functional utility for health improvising benefits.

Chapter 2, *New trends and applications in fermented beverages*, by Silvia Carballo Marrero et al., reviews the available information regarding new trends in the production and applications of fermented beverages, emphasizing their nutritional properties. Furthermore, the utilization of food industry waste products like whey or vegetable residual parts to improve probiotic properties of fermented beverages and the impact of the intake of specific fermented beverages on sports performance and recovery is also resumed here.

Chapter 3, *Fermented beverages produced by mixed cultures, pure cultures and defined co-cultures*, by Johannes Bader et al., presents some methods to control the fermentation processes of beverages in order to obtain reproducible results and standardized quality products. The development of a controlled fermentation strategy for the simultaneous fermentation of different microorganisms is described in this chapter.

Chapter 4, *New insights on winemaking of white grapes*, by Constantin Croitoru et al., describes the production and maturation of dry white wines, an important branch of still wines. The flow chart of making and aging of dry white wines is formed by the addition of selected strains of yeasts, bacteria, and nutrients extracted from the yeast cell and following stages: reception and processing of the grapes, before fermentation stage, fermentation stage, post fermentation stage and aging. Biotechnological solutions applied in the production and aging of dry white wines are also presented.

Chapter 5, *Development of red and white wines from locally adapted grape cultivars using indigenous yeast*, by G.S. Kocher et al., presents some biotechnological strategies and fermentation conditions developed to obtain traditional red and white wines with particular qualities.

Chapter 6, *An influence of different yeast species on wine aroma composition*, by Ana Jeromel et al., aims to unify the known data concerning the influence of selected yeast species in single, simultaneous, and sequential fermentations on the content and composition of aroma compounds. The combination of varietal and fermentative aroma compounds are found to be one of the first factors influencing wine quality and the choice of yeast species and selected strains are singled out as one of the determinant in defining the concentration of these volatile compounds.

Chapter 7, *Nutritional components of some fermented nonalcoholic beverages*, by Semih Otles et al., summarizes the chemical composition, nutritive properties, manufacturing technologies, and health-promoting effects of some widely consumed fermented beverages.

Chapter 8, *Fermented vegetable beverages*, by Devaki and Premavalli, discusses recent studies regarding nutrients and fermentation processes in vegetable beverages.

Chapter 9, *Fermented malt beverages and their biomedicinal health potential: classification, composition, processing and bio-functional properties*, by Muhammad H. Alu'datt et al., covers all aspects related to the processing, classification, composition, and bio-functional properties of fermented malt beverages, focusing on barley-based beverages and their potential as functional foods and for producing other high-value applications.

Chapter 10, *Kombucha tea and its health-promoting effects*, by Gülşah Özcan Sinir et al., discusses beneficial health effects and enrichment of kombucha tea with several medicinal herbal extracts as well as potential risk factors associated with the fermentation process.

Chapter 11, *Millet-based fermented beverages processing*, by Issoufou Amadou et al., discusses the bioprocessing of millet into fermented beverages, which is a biotechnical strategy with more beneficial components. Steeping, milling, slurring, sieving, fermentation, sedimentation, and cooking were the basic procedures for producing some of the fermented beverages. Malting, mashing, and fermentation of the extracts were, however, important steps in the manufacture of fermented beverages. In most traditionally fermented beverages natural uncontrolled fermentation is employed while only in few cases are starter cultures used, even though, the predominant microorganisms are lactic acid producing microorganisms, yeasts, and molds. Millet indigenous brewing technology is similar to the modern brewing technology except for the differences in the scale of level of technology used. Product obtained from millet-based fermented beverages include (Ben-saalga, Boza, Burukutu, Bushera, Doro, Fura, Jandh, Kodo ko jaanr, Koko, Koozh, Kunun-zaki, Madua Apong, Malwa/Ajon, Mangisi, Ogi, Oshikundu, Oti-oka, Shakparo Ale, Togwa, Uji, Zoom-koom, Xiao mi jiao, etc.).

Chapter 12, *Kefir: the champagne of fermented beverages*, by Jessy Van Wyk et al., highlights the most recent advances with regard to the kefir culture, the manufacturing process, and frequent applications. Apart from the unique flavor profile and the natural viscosifying effect of the kefiran produced by these organisms, kefir is widely recognized for its health-promoting (probiotic) properties, including reduction of lactose maldigestion, antitumor activity, antiallergenic effects, and therapeutic activity against colon carcinogens and arteriosclerosis. Worldwide, there is an increased tendency for kefir consumption.

Chapter 13, *Kinetic characteristics of alcohol fermentation in brewing: state of art and control of the fermentation process*, by Vesela Shopska et al., presents the main kinetic models describing the alcohol fermentation process and the accumulation of metabolites in beer production in two main areas: beer quality and control of the fermentation process. A critical analysis of the existing kinetic models for the fermentation process description, that is, yeast growth, ethanol and secondary metabolite production, and wort sugar consumption (substrate utilization), was made. Methods for process kinetic formalization were proposed in order to control the fermentation accurately with a view to optimize beer quality. The proposed studies of this paper were based on laboratory experiments and data for key biochemical foundations of the alcohol fermentation process.

Chapter 14, *New alcoholic fermented beverages—potentials and challenges*, by Ana Claudia Alencar Lopes et al., discusses some aspects related to new beverages characteristics, potentialities, and challenges, and also, aspects related to the use of selected starter cultures, focusing on the selection of yeasts to obtain improvements in the yield of the processes and increase the sensorial quality of the product. In recent decades, there has been a growing search for new technologies to produce new beverages. This search has been driven by market demand for new products and/or by the need for new ways of using alternative substrates, such as fruits other than grapes, by-products from the milk and coffee industry, and other plants like sweet sorghum. In this context, alcoholic beverages based on substrates such as fruits (e.g., papaya, orange, raspberry, etc.), cheese whey, sweet sorghum, coffee pulp, and spent coffee ground have emerged as new products in recent years.

Chapter 15, *Traditional fermented beverages in Mexico*, by Nathiely Ramírez-Guzmán et al., gives a description of more than a dozen of traditional fermented beverages made in Mexico, including tequila, mescal, stool, etc., as well as the advances and perspectives on this growing industry.

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FERMENTED BEVERAGES AND THEIR HEALTH BENEFITS

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1.1 Introduction

Fermented foods and beverages are a vital part of cultural heritage even in current time especially in Indian subcontinent. This has not taken place overnight but it evolved during the course of the history of human civilization for food preservation and sustaining the nutrition. Back in ancient times, for storage of excess foods of plant and animal origin, people adopted different preservation techniques, chiefly for the foods which are seasonal and have a short shelf life (Shiby and Mishra, 2013). Worldwide, currently, more than 5000 diverse fermented foods are consumed by menfolk. Most of them are ethnic and are produced in small quantities to fulfill the requirements of groups in a certain region. Over the course of time, fermented food preparation also expanded and included locally available diverse substrates including vegetables, grains, fish, milk, and meat products (Sekar and Mariappan, 2007). Majorly, the rural folk have reported to prefer the fermented foods over the unfermented, due to their pleasant taste, texture, and color.

In recent time, the varieties of functional foods that are available include products such as dairy foods, baby foods, confectionery, baked goods and cereals, meat products, snacks, ready meals, beverages, and spreads (Ofori and Peggy, 2013). Among them, beverages have been proved to be the most active functional food category due to it possessing several benefits such as possibility to meet consumer demands, convenience, container contents, size, shape, and appearance; better storage options for refrigerated and shelf-stable products with ease of distribution; they also facilitate great opportunities for integrating desirable nutrients, edible colors, flavurs, and bioactive compounds (Sanguansri and Ann Augustin, 2010; Wootton-Beard and Ryan, 2011; Kausar et al., 2012).

Beverages form a key part of the food industry which are intended for human consumption. Commonly, beverages are divided into

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alcoholic and nonalcoholic drinks. Alcoholic drinks include beers, wines, and spirits while soft drinks include fruit juices, tea, coffee, carbonated, and noncarbonated sweetened drinks. Milk is a natural beverage and a range of liquid milk products are produced by the dairy industry. Milk of nearly all domesticated milch animals are used in preparation of all fermented dairy products; also quite a few new types of fermented milk products are introduced and they are gaining popularity all over the world.

Fermented beverages are generally prepared from a type of sugar-containing ingredients such as cider from apples, mead from honey, sake from rice, beer from grains, and wine from grapes (the most popular beverage) (Hornsey, 2007). If one considers the primary sources of sugar available in earlier days, it becomes limited to wild berries, other fruits, tree sap, honey, and milk from animals. The traditional fermentation occurs following the activities of natural flora which is already present in the food or those added from the surroundings. Moreover, scientists have tried and succeeded to isolate and study the characters of such organisms which carry out fermentations.

1.1.1 Fermentation

Fermentation is all about providing unique flavors, textures, and other nutritional benefits to consumer products in a maximum natural way. Fermentation of beverages are classified mainly in two ways: alcoholic fermentation and fermentation of milk products.

Alcoholic fermentation, also known as ethanol fermentation, is a biological process in which yeasts bring about conversion of various sugars into ethanol and carbon dioxide and thereby obtaining the energy. Yeasts are eukaryotic microorganisms which come under the kingdom Fungi. The principal yeast species used for thousands of years in both baking and in the production of alcoholic beverages is *Saccharomyces cerevisiae* which is responsible for fermentation (Donalies et al., 2008). However, there exists a wide variety of *S. cerevisiae* strains (Legras et al., 2007), with differences in kinds of yeast recognized: wine yeast, beer yeast, mesta-yeast, growing yeast, bottom yeast, yeast juice, and yeast water (Partington, 1935). In the production of fermented beverages, ethanol is the primary product with carbon dioxide as a significant by-product. There are a large number of other minor by-products of yeast metabolism, in addition to the major products, such as ethanol and carbon dioxide (Fig. 1.1).

The fermentation of milk products is carried out into two major pathways on the basis of microorganisms involved: (1) lactic fermentation which is dominated by species of lactic acid bacteria (LAB). LABs based on their potentiality can be used in various milk products such as the thermophilic LABs in yogurt and Bulgarian buttermilk; whereas,

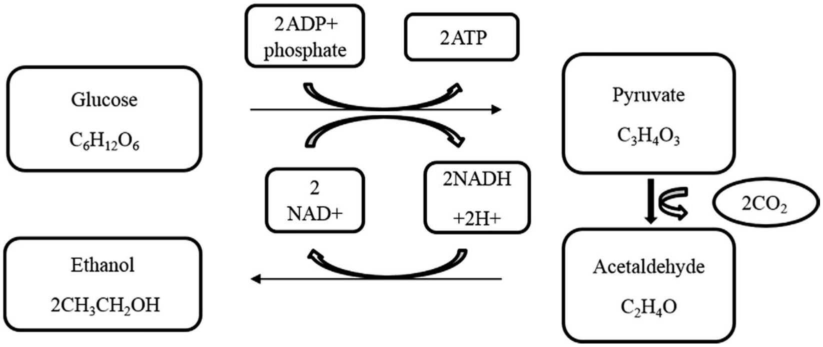

Fig. 1.1 Alcoholic fermentation process. Modified from Rasmussen, S.C., 2014. Earliest fermented beverages. In: The Quest for Aqua Vitae. Springer, Cham, pp. 13–27.

probiotic LABs in acidophilus milk and bifidus milk. The mesophilic LABs are used in natural fermented milk, cultured milk, cultured cream, and cultured buttermilk. (2) Fungal-lactic fermentations, in which LAB and yeasts coordinates to produce the final product, which comprises of alcoholic milks such as kefir, koumiss, acidophilus-yeast milk; and moldy milks such as vili (Mayo et al., 2010).

1.1.2 Classification of Beverages

Fermented beverages (Fig. 1.2) may vary depending on the type of food preparation, timing of the fermentation process, and the added microbes' application. With beer, for example, the fermentation is intentional, and typically involves a specific yeast to incorporate and enhance a desired flavor.

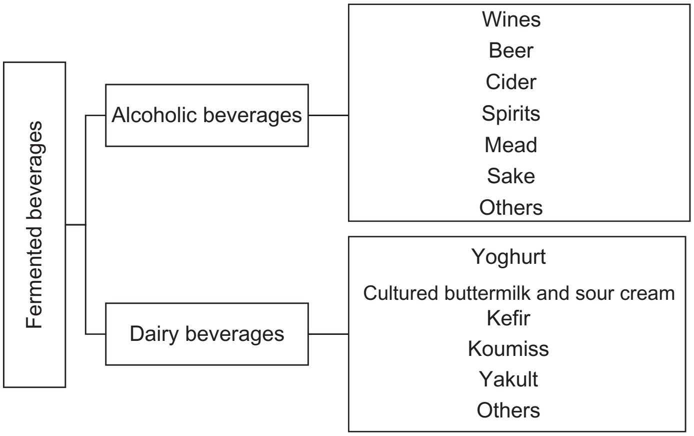

Fig. 1.2 Classification of fermented beverages.

1.2 Alcoholic Beverages

Any beverage that contains 0.55%– 76% alcohol by volume (ABV) is considered an alcoholic beverage, while others that contains more than 76% alcohol are known as medicines, for example, anesthetics. Fermentations involving production of ethanol are generally considered safe foods and beverages (Steinkrdus, 1979). Alcoholic beverages are classified as wines, beers, spirits, liquors and cocktails. These beverages are further classified based on composition, method of preparation, and amount of residual alcohol.

They are produced by two different methods: fermentation and distillation (Bieap, n.d.). Sugar, yeast, and optimum temperature are the required components for fermentation to occur, where sugar is converted to alcohol and carbon dioxide by the action of yeast. Examples of products produced by this method are beer, cider, grape wines, honey wines, Mexican pulque, palm, and jackfruit wines of India, South American Indian chicha and beers, Japanese sake, sugar cane wines, Chinese lao-chao, Thai rice wine, and Indonesian tape.

1.2.1 Wines

Wines have long been produced from all types of plant materials. In this section, wine produced from grapes will only be discussed. Wine is considered an alcoholic beverage obtained from the fermented juice of freshly harvested grapes. Although, many varieties of grapes exist, all of them are not used in the production of wine. It is prepared from both black crushed grapes and white grape juice. The quality of grapes, the color, flavor, and aroma, along with wine flavor, quality, stability, and sensorial characteristics, depends on the composition and content of some diverse groups of compounds present in the grapes. It is prepared mainly by fermentation of grapes by converting components like sugar content or part of it into ethanol and carbon dioxide by yeast. Here, generally natural yeasts on the grape skin plays a role but yeast may be added in modern industrial wine making. White wine is prepared by pressing grapes, which produces grape juice and after that fermentation is carried out. When the grapes are crushed, rose wine is prepared by fermentation from beginning. To obtain red wine, the grapes are first crushed and then fermented (Bieap, n.d.).

One should have a sound knowledge of viticulture to make wines of excellence and of sound quality in terms of color, flavor, and sweetness. Many wineries have gained experience in this field and grow their own grapes and many buy them from nearby vineyards. The temperature and climate suitable for raising wine grapes includes expectedly hot (or at least warm) days, minimal summer rain, cool nights but with minimal risk of frost, as the grapes grown in warm climate tend to lose their acidity more rapidly than those grown from cooler climate regions. Also, the soil characteristics (depth, nutrient, and moisture content) play a significant role particularly in the lesser water retention for grapes. The desirable soil should not be extremely acidic or alkaline. Mostly, rocky soil is preferable and even it is a necessity to prevent the retention of water, which has a chance to rise upwards into the vines, diluting the grape sugar or even resulting in the bursting of grapes, which in turn enables the growth of rot and mold, for example, *Botrytis cinerea*. It is known as “gray mold” by the winemakers when unwanted but “noble rot” when desirable (Bamforth, 2008). The characteristics of certain wines depend on this mold infection, for example, the Sauternes from France.

The determination of sweetness or acid balance of grapes depends heavily on the time of harvesting. Using special scissors, vintage (plucking of grapes) is often done manually. Following this, the grapes are collected in conical cane baskets and the grapes are transferred to the wine press, where they are sorted and graded manually. Ripe fruits are picked immediately before crushing and all rotten, damaged, and raw fruits are discarded. White grapes are generally chilled before crushing or picked by night. Then, the sorted and fresh fruit is fed to the destalking machine (Bieap, n.d.). The purpose of destalking is to remove stalks as they contain bitter oils and may create obstacles in obtaining desired flavor of the wine. Pinot noir is an exceptional case, which is sometimes fermented in the presence of stems for attaining its distinct peppery character. Fresh grape juice, also referred as “Must” is obtained by crushing grapes (Bamforth, 2008; Walker and Stewart, 2016) by four ways: (1) trodening, (2) balloon press, (3) manual press, and (4) electrically operated press (Bieap, n.d.). For white or blush wines, it is necessary to separate the juice from skins immediately while for red wine it is not, as the color pigments are located in the skin as polyphenolic molecules called anthocyanins, which needs to be removed immediately to prepare white/blush colored wines. Blush wines are lighter than rose wines. To prepare rose wines, contact between juice and skin during a modest fermentation (usually a few hours) is used for the appropriate extraction of anthocyanins. After rose or blush juice has been separated from the skins, the protection of Must from oxidation is necessary which occurs due to addition of sulfur dioxide (SO_2). The rapid separation of skin and juice for white wines allows minimization of grabbing of astringent tannins. This process may also impact other flavor compounds, therefore, a balance has to be maintained for certain grapes or wines in terms of SO_2 use, oxygen availability, contact time, and temperature (Bamforth, 2008).

Grapes are then moved to a drainer, a holding tank, or directly to the fermenter (in the case of red grapes). Nowadays, the vessels used for fermentation of grapes are thermostatically controlled fermentation tanks, prepared from stainless steel which are made jacketed for temperature regulation. Usually, the process of fermentation lasts for 3–4 days and temperature is maintained in the range 60–80°F. Due to vigorous fermentation, heat is produced. Excessive heat could lead to inactivation of the yeast and thus fermentation could get ceased. For preventing such obstacles, cold water is run through pipes around the tanks (Bieap, n.d.).

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

The pH during fermentation should be maintained below 3.8 as wines tend to ferment more evenly at this pH with better sensory properties and also there is a reduced malolactic fermentation. Maintaining low pH is especially important for white wines (Bamforth, 2014).

The most relevant species of yeast used in fermentation are *S. cerevisiae* and *Saccharomyces bayanus*. Dried yeast is extensively used in wine making except when produced on a commercial scale. Thus, frequent use of a specific yeast strain is of low significance. Other important factor in wine fermentations is nitrogen as a limiting component. The presence of the amino acids is a must. Addition of diammonium phosphate regularly increases nitrogen content in the process (Bamforth, 2014).

Generally, fermentation at 20–30°C takes about 2 weeks for red wine while for white wines at 10–15°C, it takes about 4–6 weeks. Fermentation process fastens at the higher temperature, accompanied with the noticeable increase in the production of flavor-active volatile substances such as esters. During the second stage of fermentation, known as secondary fermentation, bacteria converts malic acid to lactic acid, which is mild acidic in taste and is desirable (Walker and Stewart, 2016). The malolactic fermentation takes place in wooden barrels, during which the extract of various ingredients is from the wood. The progress of fermentation is monitored by measuring the decline in Brix value (Bamforth, 2014). A sweet fortified wine (a type of wine having 18%–25% of alcohol) is made by adding a definite amount of brandy. It helps by increasing the alcohol content of the wine with inactivation of yeast. Brandy is added after fermentation (when all the sugar is used up by the yeast) in process of making a dry fortified wine (Bieap, n.d.).

The process of removing unwanted materials like yeast and pulp particles from wine is referred to as racking. However, some winemakers leave the wine in contact with the yeast for several months. They feasibly use intermittent stirring. Addition of ascorbic acid sometimes prevents ‘pinking’ of white wines in the bottle. However, the chief antioxidant is SO_2, which reacts with the active peroxides in wine.

Also, addition of low levels of copper can eliminate any hydrogen sulfide present in the wine. Oxidative browning is one of the biggest threats to wine quality. After fermentation, the oxygen ingestion should be cut down or minimized (Bamforth, 2008, 2014). For racking purpose, mostly, the wines are set aside in oakwood casks with no airspace on top for about a month (so as to avoid oxidation of air which turns wine into vinegar). This will help solids to settle down to the bottom of the cask, following which the clear wine is draw off into another cask. The process is repeated several (3–4) times, thus racking can take up to 3–4 months (Bieap, n.d.).

Removal of all the suspended particles in wines is called finning. Finning can be done using any of the following finning materials: (i) egg albumen, (ii) icing glass obtained from the bladder of a sturgeon, and (iii) blue icing glass (used to remove traces of toxic copper oxide from grapes). To obtain a clear wine, these materials having clinging power are used, which attract the particles in the wine. They then settle at the bottom, following which the clear wine is dispensed off in a separate vessel. After finning, the clear wines obtained are refrigerated to avoid contamination with bacterial ingress (Bieap, n.d.). Further, filtration can be carried out if the wine is not clear enough. This process is a very slow process.

The maturation period differs significantly for various types of wines. Wines tend to benefit from aging, which is contrary to most beers. Aging is performed in tank, barrel, or in bottle. The extent of aging is less for white wines than for red ones. Wines may be matured for 3, 6, or 9 months or even for several years except champagne, which is never matured. The second fermentation for champagne and other sparkling wines takes place in the bottle itself after finning or filtration, making use of the natural sugars and yeast already present in wine, in which the presence of air in the bottle is a must. For wine, once it is bottled, it will not mature further. Usually, white wines are allowed to mature for 3–6 months and are thought to be best consumed when young. Except wines such as sweet French Sauternes, white wines are generally consumed within a few years. High-quality red wines are frequently stored for a long duration. Oakwood used in maturing being porous allows enough air into the wine, which makes the wine mallow but minimal air will not oxidize the wine into vinegar. It also makes wines smooth and removes harshness and the wine acquires color (Bieap, n.d.).

Grapes are generally the most common raw material used for wine. Particularly, in countries where no grapes are grow, other fruits berries can be used. Apples or pears fermentation yields cider. In many countries, it is considered as a common alcoholic beverage, although its consumption has declined in industrialized areas.

1.2.2 Beer

Beer is the most important alcoholic beverage produced worldwide. It can be recognized as the oldest fermented beverage. After water and tea, it is the third-most popular drink overall. Beer is manufactured by the saccharification of a source of starch (mainly barley), which is followed by extraction of the sugars which will then be further fermented by *S. cerevisiae* to produce ethanol and CO_2. The earliest known barley beer dates to ca. 3400 BC. The vast majority of beers comprise at least 90% water, with ethanol (although there are other alcohols in beer, alcohol is mostly used anonymously for ethanol) and quantitatively the second major individual component is carbon dioxide. During malting and brewing processes, extraction and digestion of starch and protein (from barley) reaches its maximum capacity which subsequently yields a highly fermentable extract that is known as "wort."

The manufacture of beer is termed as brewing. Hops (the flowers of *Humulus lupulus*) are the main flavoring agent used in beer, which contribute to a desirable bitterness and other flavors, while favoring the growth of *S. cerevisiae*; also acting as a natural preservative (Day and McSweeney, 2016). Other flavoring agents may occasionally be included such as fruit or herbs. The alcoholic content of beer ranges from 3% to 5% and is acknowledged as a very refreshing drink specifically in summer. Beer making or brewing also involve the use of germinated barley (malt). Moreover, other starch and/or sugar-containing raw materials except malt from barley can also be added, for example, other kinds of malt such as wheat, unmalted cereals known as starch flour, adjuncts (corn, barley, rice, and wheat), starch degradation products, and fermentable sugars (Bamforth, 2008).

Alcohol is derived from barley starch (which supplies most of the sugars) in the majority of beers from across the world. Barley which has high brewing value undoubtedly provides plentiful quantities of extract from the resultant malt. It comprises high starch but restrained protein content which is 9%–10%, a high degree of germination (at least 95% of kernels), and a good swelling ability. For evaluation of barley, sensory assay should also be included. Barley malting comprises the controlled germination of grain followed by drying. The raw barley kernel is hard and so not easy to mill instantly. However, enzymes developed during germination will degrade the cell walls and protein during sprouting; also, these enzymes will continue to degrade starch in the brew house. Originally, barley was selected for the brewing of beer because it has the capability to retain a husk on threshing. This forms the filter bed in most of the breweries, where separation of the extract of malt (wort) from residual material after brewing is carried out (Bamforth, 2006). Upon harvesting, the barley grain obtained is dry (almost <12% moisture) or else on account of preservation and prevention of its plague, the grains need to be dried. Usually, the water content should be increased within 42% and 46%, which is suitable for promoting germination.

Germination is stopped by driving off the moisture, which is an essential process known as "Kilning." The grain metabolism is halted and the malt is soothed for storage. Kilning must be done carefully as several malt enzymes that are useful to carry on polymer degradation in the brewery are heat sensitive. Thus, heating is provided at a comparatively low temperature to drive off the moisture. The enzymes are more stable in the drier environment when the water level has perhaps been halved, after which the temperature can be increased (Bamforth, 2008).

1.2.3 Cider

Cider is an alcoholic drink and produced by fermentation of extracts of apples. Although the term normally defines a nonalcoholic product in the United States, the alcoholic produce is termed as 'hard cider'. The biggest producer of cider is the United Kingdom (Bamforth, 2014). Also, historically the major production areas have been the counties of Hereford, West Midlands and Gloucestershire, Worcester, Devon, and Somerset, while Sussex, East Anglia, and Kent have been producing smaller amounts. Cider is similarly significant in Germany (the Trier/Frankfurt area), Northern Spain, and France (Brittany and Normandy), each of which has some distinct industrial tactics.

Cider is mostly sold as a light-flavored and clear carbonated beverage that is filled in can or bottle, having an alcohol content between 1.2% and 8.5% ABV. There is an increasing tendency toward the addition of sugars or syrups to unfermented grape must to supplement the carbohydrate derived from apple and to increase the alcohol content after fermentation known as "chaptalization." Modern ciders, for the most part, may contain only 30%–50% apple juice. In recent years, an extensive new product development has been noted, for example, paving way to higher alcohol comprising products. In the so-called "ice" versions, "white" ciders are stripped of their color, and are flavored with various added components (Lea, 2011).

Ciders tend to be noticeably sharp in flavor and lower in alcohol content particularly in France, whereas ciders from Germany tend to have a comparatively higher alcohol content. Ciders from the Asturias region (Spain) are quite foamy and vinegary (Bamforth, 2014).

Cider apples having higher sugar contents, around 15%, are said to be the best ciders. Their acidities fall within 0.1%–1%. Their shelf life can last for over a period of several weeks without losing their texture, during which period their starch converts into sugar. Their fibrous structures help making it easier for effective pressing, which will deliver higher yields of juice. Mostly, cider is manufactured not from a single cultivar but from more, so as to obtain the ideal balance of sweetness, acidity, and bitterness/astringency. It is not necessary to prepare the cider from true cider apples. Reportedly, it has also successfully been prepared from Bramley apples. The substrate which is directly derived from the apple is frequently added with apple juice concentrate also known as "AJC." Alternatively, the apple juice may be supplemented with cane or beet sugar or hydrolyzed corn syrup (Bamforth, 2014).

Nowadays, the vast majority of cider fermentations are inclined with yeast. Most of the cultures which are now added in cider were formerly isolated from the cider factories themselves. Wine yeasts with distinct characteristics are used by some cider manufacturers. Since 1980s, use of active dried wine yeast has been widespread. The temperature is likely to be within the range 15–25°C, where temperature control is affected (Bamforth, 2014).

Ciders may be subjected to malolactic fermentation, which is favored if there is no sulfiting in fermentation and storage. The oxidation of polyphenols in the juice gives rise to the color of the cider. This process can be regulated by the sulfite addition. As sulfiting is widespread these days the malolactic fermentation is probably less significant than it once was. Addition of sulfite immediately after pressing suppresses nearly all color development. Reduction of color is less, when SO_2 is added later. The standardization of the color of finished cider is carried out by the addition of caramel or other permitted colorants (Bamforth, 2014).

The traditional high bitterness and astringency of ciders originate with the procyanidins. Oxidized polyphenols get adsorbed onto the apple pulp (which makes it tanned) and this overpowers both bitterness and astringency. If oxidation occurs in the absence of the pulp, then there is a comparative shift from bitterness to astringency as the units polymerize. Alcohol has a tendency to increase bitterness but suppress astringency (Bamforth, 2014).

The yeast produces a range of volatile components (e.g., esters), as in the case of beer and wine, and the key factors affecting are yeast strain, its fermentation temperature, and nutrient composition and the clarity of the fermentation feedstock. Racking process involves removal of the freshly fermented cider from its residues. Using recent techniques, cider making may take place quite rapidly and in the absence of maturation, before blending and packaging. The cider is left on the lees for several weeks in more traditional processing, with racking into tanks for months of storage and with least contact with air. Initial clarification of cider is carried out by natural settling, by centrifugation, or by fining (chitosan, gelatin, bentonite, and in glass).

Filtration and, maybe, blending takes place before packaging the ciders, assisted by expert tasting. Most of the ciders are pasteurized and carbonated for final packaging (Bamforth, 2014).

1.2.4 Mead

Mead, or honey wine, is a fermented alcoholic beverage made from bee honey and water. Mead is produced in many countries around the world and it may be seasoned with spices (e.g., cloves, cinnamon, and nutmeg) and herbs (e.g., oregano, chamomile, and lavender). Sometimes even hops could be added, where it will be recognized as "metheglin." When mead includes fruits like blackberry, raspberry, and strawberry, it is referred to as "melomel" and products including malted barley are called "braggot." When grape juice is incorporated then we get a "pyment" (Gupta and Sharma, 2009).

Mead may be distilled or concentrated by freezing to yield honey jack (Gupta and Sharma, 2009). In the traditional manufacturing process, the honey must (unfermented solution of honey and water) is heated or boiled before its fermentation takes place for meeting the objectives of microbes elimination and protein removal (Ramalhosa et al., 2011). Modern production of mead comprises adding honey to 3–4 volumes of water with addition of desired fruits, herbs, hops, or spices. The surface froth is skimmed off after boiling, and brewer's yeast is added. Fermentation is done at 15–25°C for 3–6 weeks before aging in oak casks at 10–15°C for up to 10 years. New, gentle mead production processes without heat treatment have been developed. The ingredients added influences the content and composition of phenolic compounds in meads and also the technological processes used, for example, the means of honey must preparation, fermentation, maturing, storage in bottles, warming just before consumption, etc. and it is because these effects have been observed in the case of other similar fermented beverages such as wines (Wintersteen et al., 2005; de Simón et al., 2014).

1.2.5 Sake

Basically, sake is a rice wine. Yet production of sake is more alike to that of beer where rice starch is converted into a fermentable state before the action of yeast to produce alcohol. Typically, products contain alcohol content of around 18%–20% ABV, although this is often reduced to some 15% ABV before packaging (Akiyama, 2010). It is anticipated that sake is developed from China in the seventh century, although it is claimed that in the third century, for the emperor, the first rice wine may have been prepared. The key ingredients of sake are water and rice. Approximately 25 kL of water is used for each ton of rice.

There is much ritual and tradition in the production of sake than any other product. The water used for sake production must be colorless, tasteless, and odorless. The rice used should become sticky when cooked. Stickiness is an essential requirement here so the rice used for making sake is refined further extensively, which helps lowering the levels of objectionable lipid, protein, and minerals, than the rice employed for general food use. Sake manufacture comprises parallel fermentations, which includes saccharification of the rice starch and fermentation of resultant sugar occur simultaneously. Koji (*Aspergillus oryzae*) produces starch-degrading enzymes that generate the fermentable type of sugar and *S. cerevisiae* var. *sake*, which is known as sake yeast, converts the sugar into alcohol. During these simultaneous actions the sugars are gradually released and facilitated to the yeast so that fermentation could take place and does not expose the yeast to inhibitory high sugar levels. Rice for the premium sakes receives the most milling. White rice is reduced in weight by 25%–50% to remove the outer layers that compromise clarity and flavor. The milled grain is steeped until its moisture level reaches approximately 30%. After that it is transferred to a wooden tub which has holes at bottom to admit steam. The tub is located over a metal vessel containing boiling water for approximately 1 h. This will help to carry out starch gelatinization (Bamforth, 2014).

A share of this rice is used to make Koji (a culture of *A. oryzae*). Dried spores of *A. oryzae* are scattered over the surface of one-fifth of the rice (cooled to 30°C) and worked in. After several hours the mix is shifted to cedar trays that are shallow and covered with a cloth. These trays are then put on shelves for a maximum 2 days. To equilibrate the temperature, this mix is stirred twice every 4 h. Before pasteurizing at 60°C, fresh sake is held at a low temperature for 10 days and transferred to sealed vats which are fabricated traditionally from Japanese cedar. A 1-year storage results in a maturing of the flavor and a pickup of minutes from the wood. Thereafter, subsequent blending is done followed by tuning to final strength before bottling (Bamforth, 2014).

1.3 Fermented Dairy Beverages

The true origin of fermented milks is tough to be found yet. Although, it is presumed that they originated in Western Asia and were passed to the east. In the east region, new alternatives were established to suit the diverse climate. Fermentation of milk was helpful to humans in a way as it allowed to store and use excess milk and also making them usable while traveling long distances. Today, fermentation is used to produce dairy foods with different flavors, textures, and health benefits. Worldwide fermented milks and other related products are popular and available in many different countries. Some countries are involved in the consumption of dairy beverages and have a long history while others have developed more recently, may be due to scarcity in scientific advances.

Fermented milk is produced by the action of bacterial cultures (that generally remain present until consumption) on milk constituents (mainly milk protein), which results in coagulation of milk, without the elimination of serum. Three categories of fermented milks are defined: mesophilic sour-milks (e.g., cultured buttermilk, Viili, Skyr, Ymer, of which the fermentation is carried out at 20–30°C with lactic acid production); thermophilic sour-milks (e.g., Matzoon of which the fermentation is carried out at 42–45°C with lactic acid production); and acid and alcoholic milks (i.e., gioddu, kefir, of which the fermentation is conducted at 15–25°C with the production of some alcohol along with lactic acid and carbon dioxide) (Tamang et al., 2016; Macori and Cotter, 2018).

The microorganisms involved in the preparation of these products vary depending on the final product but frequently involve LAB, which convert lactose into lactic acid during fermentation. Various products available in the market provides a number of significant nutrients such as calcium, protein, and zinc and some of these products also claim to provide other beneficial effects particularly on the gastrointestinal tract (GIT)-related diseases. Undeniably, these products are renowned having a long history of therapeutic use in medical conditions in some parts of the world. Many products were developed commercially due to increased medical interest, early in the 20th century. Currently, one of the major areas for growth is the development of fermented products containing specific probiotic bacteria (McKevith and Shortt, 2003).

Milk and dairy beverages contain several components such as proteins, peptides, fats, oligosaccharides, vitamins, and minerals, among others which have physiological functions. To promote good health and reduce disease risk, these different milk components could be used as functional and nutraceutical ingredients. Dairy is considered to be a source of bioactive ingredients; moreover, it is also an ideal vehicle for providing nondairy functional/health components owing to their remarkable compatibility with other ingredients, superior flavor, nutritional, and bioactive profiles. Although, while integrating bioactive ingredients in dairy beverages, potentially numerous challenges are confronted by producers. Most of these challenges are correlated to the sensory attribute or physical stability of the final product that can make it undesirable to consumers.

Several different factors (majorly agricultural conditions) influence the preparation of traditional fermented milk products. Using the milk of different mammals, such as buffalo’s, cow’s, goat’s, and sheep’s milk (being the most common), cultured dairy products can be prepared. Also, the milk of other animals (e.g., yak, camel, and reindeer) is used in some parts of the world (McKevith and Shortt, 2003). Specific milks (e.g., yak's, cow's, and mare's milk) are used for the production of fermented dairy drinks. Influential factors such as environmental factors, starter and adjunct cultures, and fermentation parameters (duration, temperature, and storage vessel) together with the type of milk used, shape the microbial diversity of naturally fermented milks (Zhong et al., 2016). Mare's milk is fermented in vessels made of wooden casks or animal skin, when preparing koumiss. The filtered mare's milk is added into a vessel comprising old koumiss, thereafter, natural fermentation takes place at ambient temperature, where the old koumiss serves as the starter culture (Gesudu et al., 2016).

Key trends in functional fermented dairy beverages include major modification of the key functional ingredients such as probiotics, probiotics fiber, Omega-3 fatty acids, phytosterols, antioxidants, dietary fiber and/or changes in other nutritional factors (reduced fat, sodium or carbohydrate, and low calories), etc.

1.3.1 Yogurt

Yogurt is basically a traditional semisolid foodstuff made from milk and/or cream through the action of two particular strains of LAB: *Lactobacillus bulgaricus* and *Streptococcus thermophiles*, which share a symbiotic relationship. In some countries (e.g., Switzerland), other organisms are also used, namely *Lactobacillus acidophilus* and *Bifidobacterium* spp. (Bamforth, 2008). As per Codex, yogurt is a milk product attained by fermentation using these two specific strains with or without voluntary additions (such as other LAB, milk powder, and sugar). In United Kingdom, yogurt can be made using these strains or just one of them while there is no separate legislation for yogurt in Japan so it is not mandatory to use either collectively or separately, both the traditional strains. In Finland, similarly, for any milk products, there are also no compositional regulations. Over time, the product has progressed to a cuisine which is luxuriously diverse in flavor, with improved texture and functional properties. Therefore, the preparations may now integrate components such as grains, fruits and nuts, along with having a range of textures (Weerathilake et al., 2014).

The manufacture of fermented milk including yogurt requires the milk to be first concentrated by the addition of dairy solids which are either membrane filtered or evaporated. It can be made from skimmed (nonfat), reduced fat, or whole milk. Especially, for yogurt and other cultured milks, it is important to use good quality milk and it should be free from antibiotics and other inhibitory substances. Then the milk is heated to destroy undesirable organisms and cooled followed by starter culture addition. Yogurt products may also be added with constituents such as sugar, sweeteners, fruits or vegetables, flavoring compounds, sodium chloride, coloring stabilizers, and preservatives (Weerathilake et al., 2014). In the fermentation process, pasteurized milk that has been enriched in milk protein is inoculated with concentrated cultures of microbes followed by incubation at 40–44°C for 4–5 h. The incubation temperature should be critically maintained so that it can minimally influence the activity of the culture and thereby the properties of the finished yogurt. The yogurt bacteria produce lactic acid from lactose during fermentation, and their population rises from 100- to 10,000-fold to reach a final concentration of about 10^9 per mL. Due to the production of lactic acid, there is reduction of pH from 5.1 to 5.2, destabilization of the micellar casein occurs with complete coagulation taking place around pH 4.6. To delay the fermentation process, the coagulated milk is cooled rapidly to 4–10°C at the desired final pH.

Commercially, several different types of yogurt are available, consisting of plain (no added flavors), liquid, flavored, low lactose, and carbonated. Sundae-style is a type of flavored yogurt which include fruit puree layered at the bottom of the cup and is mixed with the yogurt before consumption. The next route which the mix takes depends on the style or type of yogurt being made. There are two general types of yogurt: stirred or Swiss-style yogurt is prepared by mixing flavors, fruit, and other bulky ingredients. Such yogurts require high levels of solids and stabilizer to obtain the desired high viscosity. For manufacturing this type of yogurt, the mix is pumped into a vat, followed by culture addition. Mixture is entirely fermented in the vat at optimum incubation temperature. Following this, the mixture is gently agitated and cooled with addition of flavor ingredients at the end of the fermentation. Then the mixture is usually pumped into final container. While, in distinction, inoculation of mix may be done using culture, following pumping instantly into the container. Fermentation directly takes place in the container. The fruit or flavoring material is first dispensed into the cup, if this fermentation style is used to prepare yogurt containing fruit or other bulky flavoring, then the yogurt mix is added on top which is followed by incubation and fermentation. The customer must practice the stirring and mixing to integrate the added flavorings all through the product. Liquid yogurts vary from gel-type yogurt to being in a pourable, homogeneous state, which are popular in Canada, Europe, and Japan. There should be no whey separation taking place during the storage (Mann et al., 2017).

Sourness is the foremost flavor of yogurt, owing to the lactic acid produced by the starter culture. Most yogurts have a pH below 4.6 and lactic acid within 0.8% and 1.0%. Most customers can distinguish sourness when the pH is below 5.0 when sweetener or added flavors are absent. Additional organic acids at much lower concentrations are also produced by the culture including formic and acetic acids. They normally make only modest additions to yogurt flavor. However, there are other metabolic products which contribute expressively to flavor development formed by the culture that accumulate in yogurt. Among them, the principal compound is acetaldehyde (which is a two-carbon aldehyde), although, a small amount normally present (at less than 25 ppm concentration) in aldehyde is still adequate to give yogurt its distinguishing tart or green apple flavor. Although, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are able to produce acetaldehyde, the rate and amount produced of aldehyde depend on the strain and on the growth conditions.

To consumers, the texture and rheological properties of yogurt are just as important as flavor. A protein gel is formed in coagulated milk which is essential to impart mouthfeel, viscosity, and body to the final product. Therefore, formation and maintenance of the gel structure is very important for yogurt quality. Various factors affect the gel properties such as the ingredients in the yogurt mix, yogurt mix processing and production conditions, culture activity, and post-fermentation, post-processing factors. Particularly in Europe, it is now common in the yogurt industry to enhance the body of yogurt by using cultures that generally produce exopolysaccharide (Lavezzari et al., 1998). Several strains of LAB can produce exopolysaccharides (mostly the thermophilic yogurt bacteria), which act as stabilizers and helps solidify the body of yogurt. Two different forms of the polysaccharides can exist: extracellular or encapsulated form. Several strains of cultures yield polysaccharides which can give ropy texture, while others offer a thickening effect without ropiness (Lavezzari et al., 1998). Criteria for sensory evaluation of yogurt generally view ropiness as a defect, so this may be important. In recent years, yogurt containing Bifidobacteria has become widespread in Canada, Japan, Germany, and France. This is produced either with Bifidobacteria in single or as mixed cultures with *Lb. acidophilus* and *S. thermophilus* and also provide yogurt with therapeutic characteristics. Human origin Bifidobacteria are preferred and they include *Bifidobacterium breve*, *Bifidobacterium longum, Bifidobacterium infantis,* and *Bifidobacterium bifidum*. Bifidobacteria are slow acid producers, so an inoculum rate of >10% has to be used. Using Bifidobacteria gives advantage in avoiding over-acidification during production and storage, and thereby giving a milder (less acidic) taste.

1.3.2 Cultured Buttermilk and Sour Cream

Buttermilk was originally produced as a by-product of butter making. It is generally the fluid remaining after churning cream into butter. It is a thin, watery liquid and rarely consumed as a fluid drink.

It contains protein, phospholipids, fat, lactose, and minerals as its main constituents due to which it is believed to have exceptional functional properties along with being a precisely good source of natural emulsifiers. Normally, it is spray dried and used as a constituent in processed food products. Buttermilk is useful to the industry in many ways as it serves as an excellent base for preparing different kinds of dressings and baked goods, to which smooth and tangy flavors are required.

Cultured buttermilk is prepared from skimmed or low-fat milk which is fermented by appropriately added LAB. Cultures for buttermilk, sour cream, and similar products must acidify the substrate and produce flavor and aroma compounds, therefore, for the manufacture of sour cream and cultured buttermilk, mesophilic multiple strain cultures are used and they comprise of two general types of organisms: acid-producers and flavor-producers. Bacteria which are citrate fermenting such as *Leuconostoc mesenteroides* subsp. *cremoris* or *Leuconostoc lactis* subsp. *lactis* var. *diacetylactis* combined with *Lc. lactis* subsp. *lactis* or *Lc. lactis* subsp. *cremoris* are typically responsible for this fermentation. In these products, diacetyl, which is the major aromatic compound can be reduced to acetoin by diacetyl reductase, therefore, cultures selected should be low in diacetyl reductase activity. During fermentation, acetaldehyde is produced often, which gives the product an objectionable "green apple" or yogurt flavor. Leuconostocs (but not lactococci) can produce ethanol from acetaldehyde, which help in flavor improvement. The use of EPS-producing starter strains is widespread in cultured buttermilk and sour cream production because their use improves the physical properties of low-fat sour cream and that of cultured buttermilk. In contrast, dairy *Leuconostoc* spp. scrutinize objectionable acetaldehyde, convert it into ethanol, which in turn provides with a complementary flavor to the overall characteristic flavor of sour cream and cultured buttermilk. A unique cultured buttermilk flavor is obtained by a diacetyl to acetaldehyde balanced ratio (necessarily) and the desirable ratio of diacetyl to acetaldehyde ranges between 3.2:1 and 4.4:1 (Marth and Steele, 2001).

The manufacturing process involves heat treatment of milk to 95°C, followed by a cooling phase up to 20–25°C, after that, addition of the starter culture at 1%–2% and the fermentation takes place for 16–20 h (Tillisch et al., 2013). The milk fat globule membranes (MFGMs) found in buttermilk is found to be rich in unique bioactive proteins (Conway et al., 2014) and is the major protein-containing constituent of this fermented product. Other buttermilk-based fermentations are combined with plants and fruits, for example, Omashikwa, which is made from tree roots (Boscia albitrunca) is a Namibian artisanal product and added to the fermented buttermilk. *Lactobacillus fermentum* and *Kluyveromyces marxianus* bacterial cultures dominate this product (Jans et al., 2017).

Although, different in appearance and texture, sour cream is actually quite similar to cultured buttermilk in several aspects such as the sour cream culture containing mesophilic acid-producing, flavor-producing, and body-forming strains, is often similar to what is used for cultured buttermilk. The flavor compounds produced by the culture and the incubation conditions are also alike for both the products. With lactic acid and diacetyl predominating, sour cream should have a similar flavor profile as cultured buttermilk.

1.3.3 Kefir

Kefir is a complex fermented dairy product which is produced through the symbiotic fermentation of milk carried out by yeasts and LAB confined within an exopolysaccharide. It is in association with protein complex known as a kefir grain. Kefir is known to provide a range of health benefits such as antimicrobial activity, tumor suppression, cholesterol metabolism, and angiotensin-converting enzyme (ACE) inhibition, as with other fermented dairy products. Moreover, it helps heal wounds speedily, and modulation of the immune system which includes the alleviation of allergy and asthma. The necessity of a kefir grain in fermentation and the presence of a large population of yeasts are the main features that differentiate kefir and many other fermented dairy products (Tamang et al., 2016).

The word kefir is derived from the Turkish word “keyif.” It means “feeling good” after its ingestion (Tamime, 2006). Its distinct flavor which is typical of yeast, and an effervescent effect felt in the mouth also characterizes the product (Rattray and O’Connell, 2011). The Caucasus Mountains are the origin of the kefir beverage. It is a traditional product highly consumed in Russia, Eastern Europe, Southwest Asia (Tamime, 2006), Middle East, and Central Asia, especially Ukraine, Turkey, Poland, Russia, and the Czech Republic, despite its lesser popularity. It is described in many reports and has been of academic interest to microbiologists because it is one of the most extensively consumed dairy products that are cultured. Moreover, it accounts for around 70% of all the cultured milk products consumed notably in the above-mentioned countries.

The beverage has low levels of alcohol, and in some cases slight carbonation and has a typically slightly viscous texture with tart and acidic flavor. Traditionally, cow’s milk is used for kefir preparation but milk from other animals such as sheep, buffalo, goat, or soy milk can also be used (Liu et al., 2006). Traditionally, in kefir preparation, fermentation was introduced by the kefir grains addition (from the formation in previous fermentation of milk) in a goat or sheep skin bag which contains fresh milk (Motaghi et al., 1997), which then will be incubated at room temperature for 24 h approximately (Walsh et al., 2016), resulting in a self-carbonated unique beverage which have yogurt-like taste properties and exotic sour test, while industrial-scale (commercial) manufacture uses bacterial starter cultures for fermentation and hardly uses kefir grains. The cultures are isolated from kefir or kefir grains with the purpose of providing more regular products (Assadi et al., 2000).

Bacterial population exist in kefir, besides typically these beverages contain plenty of other fermentation end products such as multiple volatile flavor compounds and organic acids which include the main products such as lactic acid, ethanol, and CO_2. They confer this beverage acidity, viscosity, and low alcohol content. Minor components such as acetaldehyde, diacetyl, ethyl and amino acids also contribute to the flavor composition (Guzel-Seydim et al., 2000; Rattray and O'Connell, 2011). Also an exopolysaccharide unique to kefir, known as kefiran, is produced during the fermentation process. This makes up a large fraction of the kefir grain and is also found dissolved in the liquid phase. Kefiran is a branched, water-soluble polysaccharide, contains equal amounts of D-glucose and D-galactose and is especially produced by *Lactobacillus kefiranofaciens*. Reports have shown that when *L. kefiranofaciens* grows in coculture with *S. cerevisiae*, there is stimulation in the production of this polysaccharide (Cheirsilp et al., 2003).

During the production of kefir, kefir grains function as a natural starter culture and they can be recovered by straining milk after the fermentation process is over (Rattray and O'Connell, 2011). Kefir grains are collectively made of microbes immobilized on a protein and polysaccharide matrix, where symbiotic association exist between several species of bacteria and yeast (Garrote et al., 2010). Kefir grains vary in size, ranging from 0.3 to 3.0 cm in diameter, are elastic in nature, and have a viscous and firm texture, characterized by an irregular surface and their color varies from white to yellowish white (Farnworth and Mainville, 2008; Magalhães et al., 2011). Kefir grains own a varied bacterial flora but chiefly Lactobacillus species dominate the grains although the presence of Leuconostoc, Streptococcus, Lactococcus, Pseudomonas Acetobacter, Acinetobacter along with several other species were also publicized in different grains (Leite et al., 2013; Walsh et al., 2016). Likewise, fungal microflora chiefly dominated by yeast species is also varied and species belonging to Saccharomyces, Kluyveromyces, Kazachstania, Pichia, Dekkera and Issatchenkia were stated to be present in kefir grains (Diosma et al., 2014; Marsh et al., 2013; Zhou et al., 2009). Thus, microbial species identification that are present in kefir grains is critical so as to divulge the therapeutic and technological possibilities of these microbes. Moreover, microbial species existing in grains are significant for the kefir production. Other than that volatile-compounds in kefir are also produced by these species which aid in the unique flavor properties possessed by this traditional fermented milk product. Therefore, aroma profile identification of the kefir which is formed using diverse kefir grains is important for understanding clearly the role of different microbial groups on the formation of kefir aroma which may have an impact on its final quality (Walsh et al., 2016).

Using culture-dependent techniques, generally the bacterial genera mostly found in kefir are *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Leuconostoc*. These genera have a tendency to dominate the bacterial population existing in both the milk and kefir grain, with *L. delbrueckii* subsp. *bulgaricus, Lactococcus lactis* subsp. *lactis, Lactobacillus helveticus, S. thermophilus, Lactobacillus kefiri, Lactobacillus kefir, Lactobacillus casei* subsp. *Pseudoplantarum,* and *Lactobacillus brevis* and they account for around 37%– 90% of the total microbial population present (Miguel et al., 2010). The species proportions also can differ between the grain and milk. Among the non-LAB which are associated with kefir, it is reported that *Acenetobacter* is present as one of the dominant genera in grains, often mentioned, but its presence is not reported by all research teams studying kefir. Large and variable bacterial population is present in kefir grains. Moreover, the abundant yeast population present exists in a symbiotic relationship with the bacteria. Commonly isolated three genera of yeasts from kefir grains typically contribute to the majority of the total yeast population which include *Saccharomyces*, *Kluyveromyces*, and *Candida* (Diosma et al., 2014). Upon addition of *Candida kefir* to the milk to be fermented either before or simultaneously, the growth of *Lactobacillus kefir* was reported to be enhanced. However, there was no stimulation reported in the yeast growth due to the existence of the bacteria. It is reported that when the two organisms were cultivated together, the amounts of lactic acid, glycerol, and ethanol produced were increased. Yeasts present in kefir grains are responsible for maintaining the viability and integrity of the microbial population. Amino acids, vitamins, and other essential growth factors to be required by bacteria are formed by yeasts, while yeasts use metabolic end products produced by bacteria as energy sources. The kefir grain microorganism population express symbiosis among them, which is required in the grains to sustain uniformity so that the bacteriological profile of kefir grains and the kefir drink remain stable throughout the year, where there are possible variations in milk quality and the probable presence of antibiotics and other inhibiting substances. Although, yeasts contribute to the unique characteristics of kefir, it is important to maintain the balance of bacteria to yeasts. Viscosity, pH, final lactose concentration, and the microbiological profile of the finished product get influenced by the initial inoculum concentration of the grains (grain/milk proportion) (Simova et al., 2002). Another factor affecting kefir microbial composition is agitation during fermentation. It favors the growth of homofermentative lactococci and yeast (Farnworth and Mainville, 2008; Tamime, 2006; Rattray and O'Connell, 2011). The growth of thermophilic LAB is stimulated when incubated at temperatures above 30°C, while yeast growth and mesophilic LAB are at a disadvantage (Rattray and O'Connell, 2011).

The three main ways of manufacturing kefir are: (1) the artisanal process, (2) a commercial process using Russian method, and (3) the commercial process with the pure cultures. Milk from other animal species, soybean milk, coconut milk, fruit juices, and/or sugar and molasses solutions can be used as other substrates which can be added (Rattray and O'Connell, 2011). In the traditional artisanal manufacture process, milk is inoculated with a variable amount of grains and fermentation for a period within 18–24 h at 20–25°C. The grains are sieved at the end of the fermentation process and can be used in a new fermentation batch or kept for 1–7 days in fresh milk, while the kefir beverage ready for consumption is stored at 4°C (Farnworth and Mainville, 2008; Otles and Cagindi, 2003).

In the second method known as the "Russian method," kefir is manufactured on a larger scale which uses a process of series fermentation, from the permeate resulting from the grains' first fermentation (fermentation without the mother culture or grains) (Rattray and O'Connell, 2011; Farnworth and Mainville, 2008).

All the diverse methods used for kefir production in the industrial process follow same principle. Here, the milk is inoculated with pure cultures isolated from kefir grains and commercial cultures (Rattray and O'Connell, 2011; Tamime, 2006). The maturation phase performance is not a necessity, which consists of maintenance of the kefir at 8–10°C for up to 24 h. This will facilitate microorganism, especially yeast growth, contribute to the precise flavor of the product. Omission of this step is associated with development of atypical flavor in kefir (Beshkova et al., 2002; Rattray and O'Connell, 2011).

1.3.4 Koumiss

Koumiss is an acidic-alcoholic cultured milk and it has important commercial and therapeutic significance in Russia (Kosikowski and Mistry, 1997). Mare's milk is used in its preparation. Koumiss is milky-gray in color and effervescent, with a sharp alcohol and acidic taste. Koumiss (kumys or kumiss), considering its nutritive and therapeutic properties, which is also a fermented equine milk, is broadly consumed in Central and Western Asia (e.g., Kazakhstan, Mongolia, and Kyrgyzstan) and Russia. The name koumiss is derived from the Kumanese tribe, who along the Kumane river, survived in the Central Asian steppes until approximately 1237 and it has been manufactured since approximately 2000 BC and was drunk by the initial herdsmen, the Aryans, of Central Asia (Uniacke-Lowe, 2011).

Koumiss remains a fluid even after the fermentation is complete unlike most other cultured milk products, and form coagula of different consistencies. Koumiss prepared using mare's milk does not coagulate at the isoelectric point of casein and therefore, it is not a curdled product which may contain about 1.0%–2.5% ethanol and 0.7%–1.8% lactic acid (Vedamuthu, 1982).

Generally, using chunk of the previous day's product as an inoculum, which contains a mixture of yeasts and bacteria, fresh raw milk was inoculated and thus traditional koumiss was prepared. A leather sack known as a "turdusk" (also called a "burduk" or "saba") was used to hold the milk, and it has a capacity of 25–30 L. A mixed microbial population identified in the 1960s took within 3–8 h for fermentation, which comprised primarily *L. delbrueckii* subsp. *bulgaricus*, *Lb. casei*, *Lactococcus lactis* subsp. *lactis, Kluyveromyces fragilis*, and *Saccharomyces unisporus*. More equine milk is regularly added during the agitation and maturation phases of manufacture to regulate the alcohol and acidity levels. The presence of too much yeast or excess acidification result in poor control of the whole process and often produced a product with an unpleasant taste (Uniacke-Lowe, 2011).

Nowadays, more standardized protocol is followed in manufacture of nontraditional Koumiss and other fermented milk products and significant interest has been taken for enhancing the market and consumption of these milk products in countries where it has not usually been consumed. In koumiss production, pasteurized milk along with pure cultures of lactobacilli, such as *Lb. delbrueckii* subsp. *bulgaricus*, and yeasts are used. Additionally, other microorganisms such as *Torula* spp., *Candida* spp., *Lb. lactis* and *Lb. acidophilus* can also be used in koumiss manufacture (Tamime et al., 1999; Uniacke-Lowe, 2011). When lactic and alcoholic fermentations progress simultaneously, the features of good koumiss are optimum and therefore, the fermentation products happen in certain amounts. Volatile acids and other compounds formed along with lactic acid, ethanol, and CO_2 are considerable for taste and aroma. Approximately 10% of the milk proteins are hydrolyzed. Generally, three categories of koumiss are recognized based on varying amounts of lactic acid and ethanol production: mild, medium, and strong. Koumiss is said to contain about 90% water, 1%–1.3% fat, 2%–2.5% protein (1.2% casein and 0.9% whey proteins), 4.5%–5.5% lactose, and 0.4%–0.7% ash, apart from the microbial fermentation end products (Uniacke-Lowe, 2011).

1.3.5 Yakult

In 1978, yakult launched a fluid yogurt called MilMil™ in Japan, which contained *B. breve*, *B. bifidum*, and *Lb. acidophilus*. In over 25 countries, yakult is sold globally. The presence of probiotics in isolation from other functional ingredients does not carry functional food status, therefore, in Japan, yakult is not regarded as a functional food. At the Microbiological Laboratory of the Faculty of Medicine at Kyoto University, Kyoto, Japan, Minoru Shirota (1899–1982) isolated *Lb. casei* strain Shirota (LcS) and cultured stably. The strain is found to be resistant to gastric acid and bile acids, therefore the live bacteria are able to reach to the lower intestine after oral ingestion. Yakult was developed by Dr. Shirota which is a dairy product produced using LcS, first produced in 1935. It is based on his proposition that daily oral intake of LAB help promoting intestinal health and also prevents occurrence of diseases. Thus, prolongs the life span of humans. Yakult that contains more than 15 billion living LcS per bottle has been sold for over 70 years in Japan. In 2006, about 25 million bottles per day were used up in 26 countries and terrains. Yakult is not considered as a food or a pharmaceutical. The Japanese company Yakult Honsha has prolonged supply into Luxembourg, Belgium, the United Kingdom, and Germany during 1995 and 1996 after establishing a European production base in The Netherlands in 1994 (Wright, 1999).

The popular fermented milk yakult in Japan is made with *Lb. paracasei* sp. *paracasei* strain Shirota. It contains high levels of sugar (14%) and low milk solids (3.7%) and made using a special strain of *L. casei* (strain Shirota) that was originally isolated from the human intestinal tract and is marketed in 65-mL bottles containing 6.5 billion bacteria.

1.3.5.1 Other Fermented Dairy Beverages

Acidophilus milk is a milk product prepared involving the probiotic bacteria *L. acidophilus*, which is a microbe from the flora of the normal GIT. This and other correlated products (such as Acidophilus yogurts) are widespread in the United States. Laban is a cultured milk drink. It is usually based on cow's milk and consumed in many Gulf countries. Traditional laban, which is homemade contains a mixture of LAB, molds, and yeasts. Three strains of LAB are used in its commercial preparation. Similar products are available in Tunisia, Egypt, and Morocco. Lassi is a yogurt drink originally from East India that can also be sweetened and flavored with fruit or rosewater. Popular in Scandinavia, ropy milks are prepared using starters that produce slime (mucus), which provides a unique viscous quality to the final product (McKevith and Shortt, 2003).

Some fermented products can also be made using grains, coconut milk, soya milk, and rice as a base material, for example, boza, a nonalcoholic drink made from cereal (such as maize, wheat, rye, or millet) which are some of the nondairy ingredients and widely consumed in Bulgaria and the Balkan peninsula. Direct acidification using organic acids can be used to prepare acidified milk products (such as sour cream) (McKevith and Shortt, 2003).

Ayran is a refreshing beverage. It is prepared simply by dilution of 1/3 yogurt with 2/3 water (occasionally milk), outdone with chopped mint or other aromatic herbs, salted lightly and carbonated or non-carbonated. It has a sour and mildly salty flavor with creamy and smooth mouthfeel. It has added sugar and is nonalcoholic. Ewe's milk yogurt is the base for making best ayran and is best served chilled. In Turkey, ayran has gained very much popularity and has recently been declared the national summer drink (Shahidi and Alasalvar, 2016).

1.4 Health Benefits of Fermented Beverages

Fermented milks and other related products may have other benefits above and beyond their nutritional content. Fermentation stage in fermented milks and related products preparation affect the nutritional composition of the milk or base constituent from which they are prepared. Mostly, milk has been serving as a good source of a wide variety of nutrients in milk-based products. The nutritional composition of the fermented final product habitually differs from milk attributable to the changes that take place during fermentation. Fermented products are in general more digestible compared to unfermented ones. Therefore, in several societies fermented products are used as weaning foods. Acid is produced slowly, allowing small particles to form during fermentation. It also helps increasing the surface area for later human enzyme digestion. Before the recognition of the existence of bacteria and other microorganisms, traditional products (such as laben from Syria) have been consumed therapeutically in health disorders of the intestine, stomach, and liver, and for stimulating the craving for decades. In Russia, therapeutically, both kefir and koumiss are consumed.

In the case of probiotics addition mainly in milk products, within the human GIT, specific probiotics may show a parallel effect. They do so either by changing the environment (mostly reducing the pH) or by colonization resistance (which prevents other microorganisms esp. pathogens from adhering to the GIT). Thus, they help decrease the occurrence and/or interval of intestinal infection. Bacteriocins, the substances produced by some probiotic bacteria are toxic to other (mostly related) strains of microbes, which also has been established. Other probiotic components may also provide advantageous effects on undesirable microbes, for example, recently, a supernatant of one probiotic has been shown to downregulate *Helicobacter pylori* infection in humans (Patel et al., 2014).

Numerous health effects for probiotics have been established, which include of alleviation of the symptoms of lactose intolerance, decrease of period of rotavirus diarrhea, decrease in detrimental intestinal microbial enzyme activities, diminished fecal mutagenicity, and immune improvement. Although, it cannot be presumed that all probiotic strains of even a definite species have the same or even any required properties. The consumption of fermented dairy products is generally low in several countries where yogurt has not been taken as part of the regular diet.

The fermented milks and related products seems in growth area for being in the value added and health sector of the market–functional, probiotic, and fortified products. Several factors will affect the acceptance and further growth in the market, including the emergence of more specialty products, progress of fermented products using plant material, quality and sensory improvements, genetically engineered strains, and continued or increased interest in "healthy" foods.

According to Marquina et al. (2002) kefir consumption significantly increased LAB counts in the intestinal mucosa and reduced Enterobacteria and Clostridia populations. It also prevented *Campylobacter jejuni* colonization in chick ceca (Zacconi et al., 2003). It was also found effective in patients with gastrointestinal disorders and in postoperative treatments (Sarkar, 2007). Researchers have used kefir in Russia in the treatment of peptic ulcers in the stomach and duodenum of human patients (Farnworth and Mainville, 2008). The induction of apoptotic cell lysis in tumors, the inhibition of tumor growth, and substantial rises in IgA levels in mice suggested that kefir endorses resistance of the mucosa to intestinal infections and possibly has antitumoral properties by Liu et al. (2002).

Compared to other alcoholic beverages, red wine consumption may exert an additional protective effect against CVD. In a study, 10 healthy males were given dealcoholized red wine, red wine, or gin for 20 days and the outcomes disclosed that non-beneficial bacteria from the human microbiota can be inhibited by red wine polyphenols and potentiate the growth of probiotic bacteria such as Bifidobacteria, which could be implicated in the reduction of CRP and cholesterol.

1.5 Conclusion

Milk comprises plentiful bioactive components both in the skimmed part and in the lipid, hence, is considered a rich source of nutrients. The passion to value addition to dairy and advances in new processing technologies have stimulated substantial commercialization of healthy bioactive components from milk. Functional constituents such as colostrum, lactoferrin, GMP, and protein hydrolysates from dairy have been fruitfully commercialized and discovered applications in infant formula, immune and antibacterial enhancement, and sports nutrition products. In controlling food consumption and obesity-related metabolic ailments, milk and fermented milks, including koumiss, kefir, and ayran, are known to regulate the satiety. The alcohol containing products should be consumed mildly so as to avoid the brutal consequences. Although milk and dairy products persist as ideal vehicles for the transfer of bioactive constituents, manufacturers face a number of potential challenges when integrating bioactive components in dairy beverages. Although not impossible to overcome, but most of these associated to the physical constancy or sensory characteristic, can make the final product objectionable to clients.

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NEW TRENDS AND APPLICATIONS IN FERMENTED BEVERAGES

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2.1 Fermented Beverages, Definition, and Importance on Human Diet

Fermentation is a technique used for human societies since ancient times due to their desirable effects of extending shelf life of products and modifying organoleptic properties. Today, the industry of fermented beverages is gaining attention because consumption of traditional products continues and new industrial beverages are gaining interest. The preservative properties are due to the ability of the fermenting microorganism to inhibit the growth of the pathogenic microorganism. Actually, the inhibitors are products of the fermentation process such as organic acids, hydrogen peroxide, and bacteriocin which produce an unfavorable environment for the growth of pathogenic microorganisms (Borresen et al., 2012). In addition, the organic acids gave the typical acid taste of some beverages, like yogurt among others.

From a scientific view, fermentation is a desirable chemical transformation of organic complex components of foods or beverages into simpler compounds by the activity of certain enzymes in the bearing of some specific microorganism. During the process, microorganisms obtain energy for their affairs and food for life. Meanwhile, the enzymes and other products of the fermentation are produced in situ by microorganisms such as yeasts, molds and bacteria. These enzymes cause a relevant function over the digestibility of the foods and the bioavailability of nutrients in humans after digestion. They can have

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different functions as breaking proteins and peptides in amino acids, carbohydrates into simple sugars, and fats into free fatty acids.

Nowadays, fermented foods in general and specifically fermented beverages are gaining attention internationally. Not only for their preservative benefits mention before but also for the additional properties that this process can add to the resultant fermented products such as enhancement of nutritional value and better digestibility of some components (due to lipases, proteases, and amylases that are formed during fermentation), increase of nutrients such as vitamins, fatty acids, or greater bioavailability of polyphenols and other bioactive substances (peptides, exopolysaccharides, phenolics, and neurotransmitters), reduction of undesirable compounds (phytates, tannins, etc.), inhibition of pathogens, and so on. Some of these components are strain dependent such as B-vitamins that are synthesized from nonvitamin precursors by specific bacteria and certain polysaccharides. In addition, most of these substances that are formed or destroyed in the fermentation process are responsible for the health benefits of fermented products of the original ingredients (Marco et al., 2017). Precisely, these health benefits are the precursors for the increasing worldwide demand for fermented products in the last years.

The increasing evolution in the interest of consuming and developing beverage fermented products with health implications is reflected by the increasing tendency in manuscripts published in the last years that appear cited in the WEB of Science when a search with the terms “fermented beverages” and “health” was done. Fig. 2.1 shows that from the 174 registers that appeared in the search, most of them were published in the last 5 years.

Most of the health beneficial effects cited in the chapters in bibliography for fermented products are the antimicrobial activity, antitumor via inhibition of mutagens in vitro, reduction of carcinogen-generating faecal enzymes in vivo, stimulation of the immune system, suppression of tumor formation, reduction of cholesterol levels, cardiovascular disease, type 2 diabetes, and so on (Chen et al., 2014; Tapsell, 2015). These beneficial effects are mostly attributed to the consumption of lactic acid bacteria (LAB) (Steinkraus, 1994) but there is still a lot of research to do trying to explain whether there is enough scientific evidence for most of the beneficial effects declared for some of these fermented products.

Even though the advantages of consuming fermenting beverages are many, there are also some risks which cannot be undervalued. The acid pH and other preservation practices sometimes are not sufficient and contamination with pathogens in fermented foods can occur during any step of the production and/or during the preservation process. One of a fatal pathogen is the botulin produced

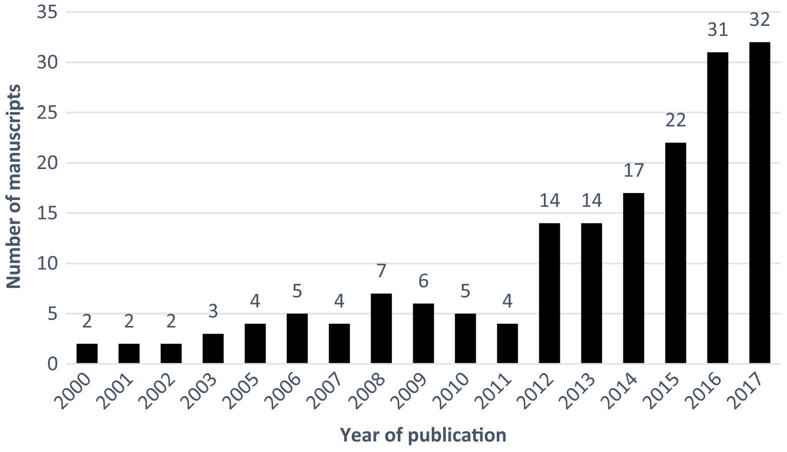

Fig. 2.1 Number of publications per year in the WEB of Science for a search with the terms "fermented beverages" and "health". (Search done: 2nd February 2018).

by *Clostridium*, which is associated with food spoilage. Another one is salmonella that can also be found in some fermented products. Because of the fermentation process, toxic molecules can also be produced naturally and their concentration must be controlled to reduce its potential toxicity. An example of these toxic molecules is the biogenic amines. These compounds can be generated during fermentation by microbial decarboxylation of amino acids. The bacteria responsible for the decarboxylation reaction possess amino acid decarboxylase activity. That type of bacteria is present naturally in unpasteurized milk that is why some cheese made with untreated milk present high quantities of some biogenic amines, meanwhile the presence in yogurt and other milk beverages have little presence or nondetectable amounts (Simon Sarkadi, 2017). This is why biogenic amines can be found in dangerous quantities in alcoholic drinks such as wine and spirits. Another potential risk is the presence of mycotoxins such as aflatoxins in ingredients like legumes, nuts, and cereals. For that reason, strict control of the risk factors during the processing of the beverages has to be taken in order to take advantage of the beneficial properties that fermented beverages have (Wilburn and Ryan, 2017).

2.1.1 Worldwide Consumption and Production of Fermented Beverages

By continents, Africa is the continent with the highest consumption of fermented beverages as there is a long tradition of consuming these wares with an average of 23 kg per person per year. Furthermore, the production of these beverages in Africa is also the highest compared to the rest of continents, around 20,000–23,000 miles of tons per year according to the FAO Database (Food and Agriculture Organization of the United Nations, n.d.). This elevated production is due to the fact that in Africa the poor processing and storage conditions contribute to contamination of fresh milk. For that reason to avoid contamination problems, fermented sour milk is widely distributed in Africa (Jans et al., 2017). The other four continents show much lower tradition on consumption of fermented drinks being Europe the second continent in consumption and the United States the last one (see Fig. 2.2).

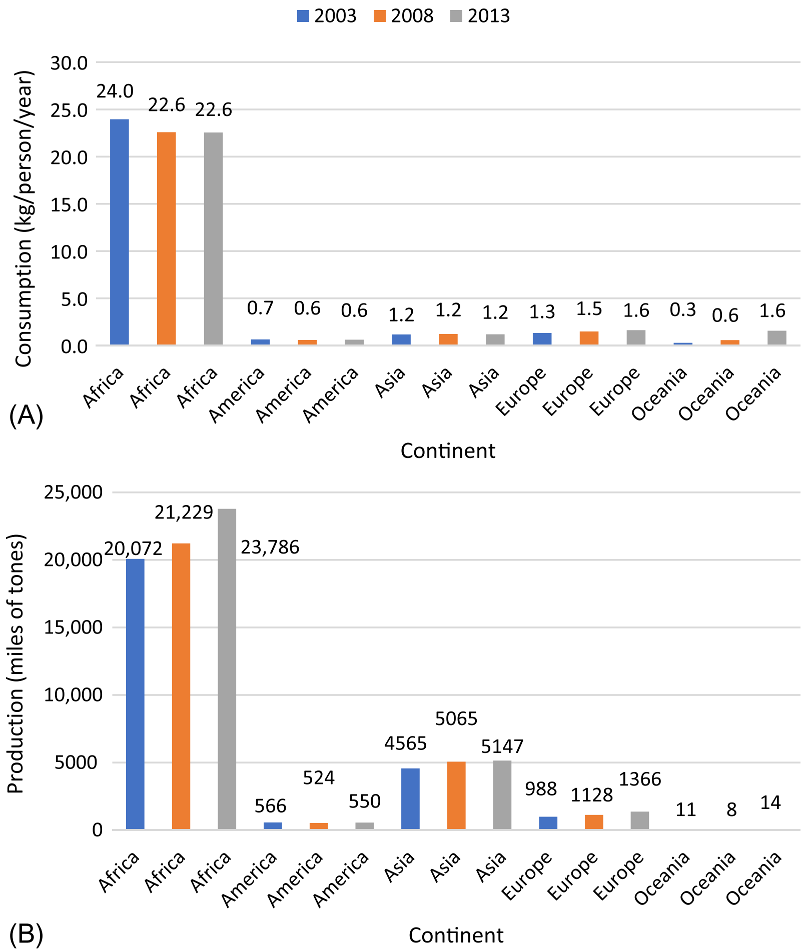

Fig. 2.2 Data on production (A) and human consumption, (B) corresponding to the five continents during the years 2003, 2008, and 2013. Source: Food and Agriculture Organization of the United Nations. FAOSTAT Statistics Database. Available from: http://www.fao.org/faostat/en/#data/FBS (Accessed 15 September 2017).

It should be noted that Oceania is the continent which is suffering a higher increase in consumption of these products in the last years but it is still, the continent with the lowest production data as there was no tradition of consumption of fermented beverages.

2.1.2 Origin of Beverage Fermentation

The exact origin of the process is unknown but it seems that lactic acid fermentation was the first one to be used as there is an evidence of being employed for the first time to produce cheese 8000 years ago in the fertile rivers of Tigris and Euphrates (now, Iraq) simultaneously to the domestication of animals and the development of agriculture (Fox and McSweeney, 2004). The origin of fermented beverages is also known to be quite antique as different civilizations left archaeological evidence of the use of that sort of fermentation to preserve food and drinks in ceramic containers. The first alcoholic fermented beverages appear to be associated with the discovery of alcoholic fermentation. It seems that the first alcoholic fermented beverage made of rice, fruit, and honey was prepared by the Chinese and Georgian 7000 years ago (McGovern et al., 2004). A bit later, 6000 years ago, the Egyptians developed the procedure to elaborate wine from grape juice. On that point, there are also evidences that Babylon, pre-Columbian Mexico, and Sudan were also using fermentation for obtaining beverages 5000, 4000, and 3500 years ago. For example, Pulque that is an ancient alcoholic beverage made of agave juice drank by the Aztecs in South America. Moreover, beer beverage was first elaborated 3800 years ago by the Sumerians using barley as the principal ingredient. After those first alcoholic fermented beverages, quite a lot different varieties of alcoholic fermented beverages have been consumed locally as the traditional products and are still being consumed all over the world, such as sake and whiskey. Nonalcoholic fermented beverages have also an antique origin. It seems that yogurt and other fermented milk were discovered accidentally as a result of primitive methods of preserving this product in warm climates. Most historical accounts situate the origin of yogurt in the Neolithic when people of Central Asia around 8000 years ago began the practice of milking their animals. These findings appear to have been fortuitous and it was not until the nineteenth century when the advances of some scientists (Fig. 2.3) gave the basis of what fermentation consists today. For example, the first industrialized production of yogurt is attributed to Isaac Carasso in 1919 in Barcelona (Ross et al., 2002).

Pasteur chemically explained in what the fermentation consisted of. He proved that fermentation was started by living organisms. For that reason, he called this process "fermentation" that means

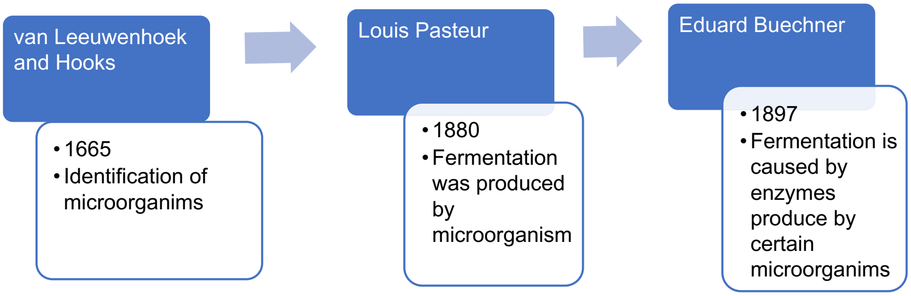
van Leeuwenhoek and Hooks
• 1665
• Identification of microorganims
Louis Pasteur
• 1880
• Fermentation was produced by microorganism
Eduard Buechner
• 1897
• Fermentation is caused by enzymes produce by certain microorganims

Fig. 2.3 Remarkable chronological scientific developments in the understanding of the fermentation process.

life without air referring to the anaerobic microorganisms (Ray and Joshi, 2014). Pasteur thought that the transformation of complex carbohydrates in simple sugars was directly due to the microorganism. Lately, in 1897, Bouecher explained that fermentation was caused by enzymes which were produced by the microorganism. Until the nineteenth century, the production of fermented beverages was mainly local but after Bouecher's discovery and due to the coincidence in time with the industrial revolution in Europe, the development of fermentation products grew on to industrial scale.

Nowadays, the resulting beverage of the fermented process depends enormously on the use of defined strain starters. The starters must guarantee to be innocuous for humans. In this line, a lot of work and research has been done since 1928 when Rogers and Whittier discovered a product of some LAB called “nisin.” They found that “nisin” has antagonistic activity against other foodborne bacterial pathogens. Since then great advances have been carried on selecting the best strains to be used for each application that assure a history safe of use or general recognized as safe (GRAS) for the final product. The term history safe of use employed for certain microorganisms must be understood as “a significant human consumption of food over several generations and an in a representative genetically population for which there exist adequate toxicological and allergenicity data to publication of a list of microbial food cultures generally recognized as safe (GRAS) to be used in all types of foods and beverages” (Bourdichon et al., 2012).

2.1.3 Types of Microorganism Employed in the Fermentation Processes

Different types of bacteria, molds, and yeasts have been used in traditional food fermentations, however, today there is a tendency to employ patented microbial food cultures for commercial purposes that have a story of safe used and nontoxicity for humans. Most of these microorganisms employed in the fermentation of beverages are bacteria and among all, the most common is the LAB. LAB are Gram-positive acid tolerant organisms that transform simple sugar in lactic acid (Borresen et al., 2012). Additional to these microbes depending on the beverage sometimes particular strains are added to impart specific properties to the final product like *Bacillus* a non-LAB. LAB comprise a large group of about 380 species in 40 genera of 6 families (Tamang et al., 2015). Each type of bacteria produces in the fermentation process different type of substances being some of them responsible for the organoleptic properties of the final beverage. The diversity in LAB strains explains the great variety of fermented products that can be found all around the world. In addition, to the LAB, yeasts can also be used to ferment sugar and produce secondary metabolites with enzymatic properties. Yeasts can also inhibit the growth of mycotoxins. And finally, molds are responsible for the production of lipolytic and proteolytic enzymes and the degradation of antinutritive factors. The components and microorganism necessary to initiate each type of fermentation, subproducts and the final products of the process are shown as a scheme in Fig. 2.4. Depending on the microbes employed, the fermentation process can be classified as:

a) *Lactic acid fermentation* occurs when LAB carry out the conversion of carbohydrate to lactic acid plus other substances without the need of oxygen. Depending on the type of LAB, two different carbohydrate catabolism pathways can occur. Homofermentative species of LAB convert sugars in milk mostly into lactic acid via the glycolytic pathway (i), whereas the heterofermentative species convert sugar into lactic acid, acetic acid, ethanol, and CO_2 via the 6-phosphogluconate pathway (ii) (Fig. 2.4). Production of

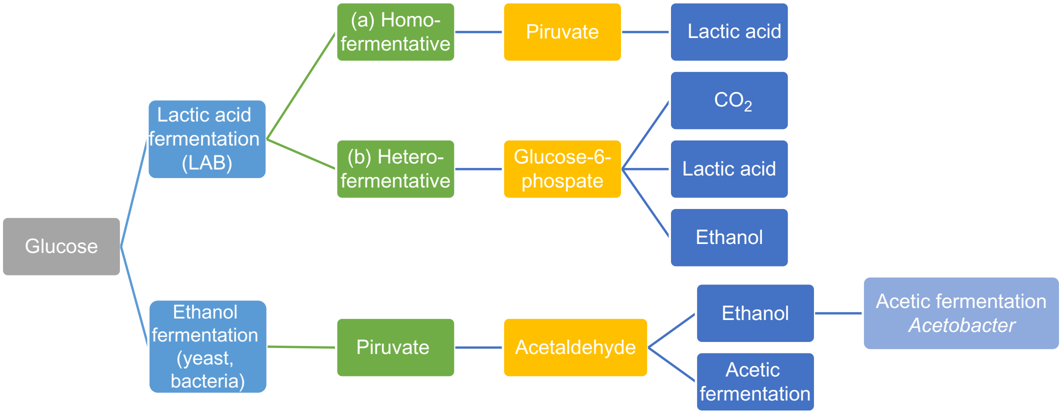

Fig. 2.4 Lactic acid, alcoholic, and alkaline fermentation pathways.

lactic acid by the LAB is strain dependent, for example, the most common bacteria belonging to the homofermentative group are *Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus*, and some *Lactobacilli,* meanwhile, the mean representatives of the heterofermentative group are *Leuconstoc*, *Weissella*, and some *Lactobacilli* (Macori and Cotter, 2018).

b) *Alcoholic fermentation*: This fermentation is produced by yeast without the presence of oxygen. These microbes have the capacity of degrading malic acid into ethanol and CO_2. *Saccharomyces pombe* and *S. boulderi* are the dominant yeasts used in the production of fermented beverages. Beer is produced by *Saccharomyces cerevisiae.*

c) *Acetic fermentation*: The oxidation of alcohol into acetic acid in the presence of oxygen by the agency of bacteria of the genus *Acetobacter*.

d) *Alkaline fermentation*: Not as usual as the three before mention. In this fermentation, Bacillus species (*Bacillus subtilis*, *Bacillus pumilus*, etc.) are used to hydrolyze the proteins and releasing ammonia which increases the pH. This sort of fermentation is common in rich protein ingredients such as soya, etc.

2.1.4 Health Claims Categories for Fermented Beverages

In addition, to the type of fermentation, it is important to know the type of microbiota employed as not all these microorganisms can be considered as “probiotics.” The FAO/WHO gave the following definition of “probiotics” in 2001: live microorganisms which when administered in adequate amounts confer a health benefit on the host (Bielecka, 2006). Their proposal of definition for “probiotics” was slightly reworded by the experts of the International Scientific Association for Probiotics and Prebiotics to “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.” According to the experts, this definition includes a broad range of microbes and applications and it is necessary to clarify the differences between live microbes used as processing aids or sources of useful compounds from those that are administered primarily for their health benefits (Hill et al., 2014). This is not an easy task as it is not always possible to clearly distinguish the contribution of live microbes from that of the food matrix in most of the studies. In addition, most of the possible beneficial microbes represent a not defined strain in terms of composition and stability. For that reason, they cannot be considered “probiotics” as well.

As a result of the study, the International Scientific Association for Probiotics and Prebiotics recommended to describe those foods as “containing live and active cultures” to differentiate them from probiotics, they also recommended that the term probiotic to be used only on products that live microorganisms with a suitable viable count of well-defined strains with a reasonable expectation of delivering benefits for the well-being of the host (Marco et al., 2017). As a guide for consumers and producers of fermented beverages, the panel of experts defined some categories of health claims for live microorganism for human use which are shown in Table 2.1 (Ricci et al., 2017).

In the table, apart from the description of the product, the criteria and a minimum level of evidence to give the claim is also given.

For fermented beverages, the categories of claims are three: (a) beverages with live active cultures; (b) beverages that contain probiotics but not enough evidence of a beneficial effect in humans; and (c) beverages with microbes of which there are enough evidence of a beneficial effect and specific claims can be given on the label of the product. The type of microorganisms that can be used as probiotics when producing beverages varies with the time, but usually are bacteria, yeast, or mold. However, the most common microorganisms used as probiotics are (Amara and Shibl, 2015):

Bacteria (Kandylis et al., 2016)

- *Lactobacillus*: *acidophilus, sporogenes, plantarum, rhamnosum, delbrueck, reuteri, fermentum, lactus, cellobiosus, brevis, casei, farciminis, paracasei, gasseri, crispatus*
- *Bifidobacterium*: *bifidum, infantis, adolescentis, longum, thermophilum, breve, lactis, animalis*
- *Streptococcus*: *lactis, cremoris, alivarius, intermedius, thermophilis, diacetylactis*
- *Leuconostoc mesenteroides*
- *Pediococcus*
- *Propionibacterium*
- *Bacillus*
- *Enterococcus*
- *Enterococcus faecium*

Yeast and molds:

- *S. cerevisiae, Saccharomyces boulardii, Aspergillus niger, Aspergillus oryzue, Candida pintolopesii, S. boulardii*

Nowadays, it is a great challenge to include probiotics in beverages as bacteria need to survive during the shelf life of the product and the gastrointestinal digestion while supporting desirable nutritional and functional properties. Fostering the growth of stress-tolerance strains to extreme environmental conditions—food matrix, oxygen level, pH, storage temperatures, postacidification at the storage stage, gastrointestinal conditions—during the shelf life of fermented products is key to assure survival of bacteria and, hence, their potential beneficial effect over the host health (Gueimonde and Sánchez, 2012). For that reason, to assure the health benefits, it has been recommended

Table 2.1 Categories of Possible Health Claim Employed in Fermented Beverages Depending on the Microbes Added as Regards to the Expert Panel of the International Scientific Association for Probiotics and Prebiotics

Claim in the Label	Description of the Product	Criteria	Minimum Level of Evidence Required to Make Claim	Comments
"Contains live and active cultures"	A beverage with live active cultures	Any food microorganism. Proof of viability at a minimum level (suggested to be 1×10^9 CFU per serving) (Bertazzoni et al., 2013)	No specific efficacy studies for a product needed	
"Contains probiotics"	Probiotic in a beverage without a health claim	A member of a safe species, which is supported by enough evidence of a generally beneficial effect in humans. Proof of viability at the appropriate level used in supporting human studies (Ricci et al., 2017)	Evidence with enough human studies supporting the observed general beneficial effect for the taxonomical category of microbes concerned. The evidence could not be generated for the specific strain included in the product	Extrapolation of evidence must be based on reasonable expectations that the strains incorporated in the product would have similar general beneficial effect in humans
''Helps to reinforce the body's natural defences in children" or "helps reduce the risk of antibiotic-associated diarrhea"	Probiotic in beverages with a specific health claim	Defined probiotic strains.	Convincing evidence needed for specific strain or strain combination in the specify health indication	Well-designed observational studies are used to detect the effects of foods on health. Samples sizes must be large enough to manage confounding factors

Adapted from Hill, C., et al., 2014. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11, 506–514.

of these bacteria that it is present at the expiry date of the beverages in a minimum level of 10^6–10^7 CFU mL^{-1} (Shori, 2016). Moreover, it is also a matter of research the development of symbiotic beverages. Symbiotic is defined as the combination of probiotics and prebiotics (the food components they eat) in one food or beverages.

2.1.5 Types of Fermented Beverages Depending on the Ingredients Employed in the Production

Because of the enormous quantity of strains and ingredients that can be employed in the fermentation processes, there are a great variety of fermented beverages in the market. They are sometimes classified as dairy beverages and nondairy beverages. Dairy fermented beverages are, the more traditional ones and in continents such as Africa, there are a great variety of traditional products depending on the zone, but in the last years, a great development in nondairy fermented drinks has been done trying to find solutions for vegetarian people and two different kinds of intolerances or allergies to a beverage component. Among nondairy beverages, fruit, vegetable, and cereal nature are the ones which are found in great quantities in different regions. Most of them are nonalcoholic beverages, but others are alcoholic worldwide-known beverages such as beer and wine.

2.2 Fermented Milk Products

The Codex Alimentarius defines the CODEX STANDARD FOR FERMENTED Milk a *fermented milk* as:

- "A product obtained by fermentation of milk. Milk may have been manufactured from products obtained from milk by the action of suitable microorganisms and resulting in the reduction of pH with or without reduction coagulation. The starter microorganism shall be viable, active and abundant in the product to the date of minimum durability" (Stan, 2010) (CODEX STAN 243-2010).

In addition, to fermented milk the CODEX STANDARD FOR FERMENTED Milk includes other possibilities:

- *Concentrated fermented milk*: a fermented milk in which the protein has been increased to a minimum of 5.6%. Examples of concentrated fermented milk are Stragistro, Labeh, Ymer, and Ylette.
- *Flavored fermented milk*: fermented milk containing a minimum of 50% of nondairy ingredients such as sweeteners, fruits, vegetables, and other components.
- *Drinks based on fermented milk*: fermented milk mixed with water mixed or not with another ingredient such as whey, flavorings. These products must contain a minimum of 40% fermented milk.

Fermented milk has been a part of human nutrition since ancient times. The beneficial effects of its consumption in humans have been experienced during centuries but it was not until 1910 when Elie Metchnikoff hypothesized the presence of desirable bacteria in Bulgarian milk that could help to substitute in human gut undesirable bacteria for desirable one (Koutinas, 2016). There are nearly 400 generic names of fermented milk applied to variations of this product around the world. Most of them are produced locally and fewer are commercial products internationally distributed. So, there is no sense that in this subsection to mention all varieties of products as there are quite a lot of works published dealing with this topic (Baschali et al., 2017; McGovern et al., 2004; Ray and Joshi, 2014; Tamang and Kailasapathy, 2010).

2.2.1 Classification of Fermented Milk Depending on the Fermentation Process Suffered

Fermented milk products can be classified into three groups depending on the fermentation developed: lactic acid fermentation, yeast-lactic fermentations in which yeast and LAB intervene to get the final product, and also mold-lactic fermentation. This last type of fermentation is mostly used in cheese production. The possible microbes and examples of each type of fermentation are shown in Table 2.2 (Khurana and Kanawjia, 2007). Lactic acid fermentation is the most extended fermentation process and depending on the microbes employed the milk obtained can be classified in thermophilic, probiotics, and mesophilic sour milks.

Nowadays, as mentioned before, it is very important to maintain alive most of the microorganism used in the fermentation process to keep active its beneficial effects. The health benefits derived from the consumption of beverages containing *L. acidophilus* and *bifidus* are well known, that is why numerous products are commercialized worldwide. In dairy products, there are a lot of factors that can influence in the viability of the microbiota such as pH, the presence of hydrogen peroxide and oxygen, the concentration of acetic and lactic acids, storage temperature, nature of additional ingredients, and the strains employed (Shori, 2016). All these variables should be optimized in the development of a fermented beverage to obtain the desired effect. For example, it is known that probiotic bacteria need certain compounds for their growth which are missing in milk, for that reason they are usually added to the milk. Some of those compounds added in industrial fermented milk products are fructooligosaccharides (FOS), caseinomacropeptides, whey protein concentrates, tryptone, yeast extract, nucleotide precursors, and iron (Janer et al., 2004). The importance of biopeptides lies in the relevant physiological functions

Table 2.2 Types of Fermented Milk Based on the Microorganism Employed

Dairy Milks	Type of Microbes	Products	Min. Composition (CODEX Standard for Fermented Milk)	Substrate	Country of Production
Lactic acid fermentation	Thermophilic (*Lactobacillus delbrueckii* subsp. *Bulgaricus*; *Streptococcus salivarius* subsp. *thermophiles*; *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, *Lactobacillus acidophilus*)	Yogurt Bulgarian Buttermilk Acidophilus milk	a min of 2.7% protein and less than 10% fat	Cow and goat milk boiled	Russia, Bulgaria, Hungary
	Probiotic	Acidophilus milk Bifidus milk		*Cow milk*	
	Mesophilic (*Lactobacillus lactis*)	Naturally fermented milk Cultured milk		All types of milk	
		Kefir	a min. of 2.7% protein and less than 10% fat	Any kind of milk	Mainly in Eastern Europe
Yeast-lactic fermentations	LAB and yeasts	Koumiss	less than 10% fat min. 0.5% ethanol (vol./w)	Cow but also other milks	Turkey, Cyprus, Greece, Macedonia, Bulgaria
Mold-lactic fermentation	Molds (*Geotrichum candidum*) and LAB	Viili			Sweden and Finland

that they provide to humans such as antihypertensive, antimicrobial, immunomodulatory, anticancer, antithrombotic, etc. The release of peptides during the manufacture of fermented milk beverages can be due to the microbial present with proteolytic action and also by endogenous proteolytic enzymes (Martinez-Villaluenga et al., 2017). Not all peptides have the same bioactivity, it depends on the amino acid composition, the sequence, and size. An interesting review was written by Martinez-Villaluenga et al. on a recompilation of different bioactive peptides encountered in animal origin fermented foods was shown. The most commercially and consume dairy fermented beverages will be commented briefly in the following lines.

2.2.1.1 Fermented Milk Obtained After Lactic Acid Fermentation

All these products are obtained when the pH of the milk lowers (pH < 4.6) as a consequence of the appearance of lactic acid causing the coagulation of proteins without the elimination of the serum. At that pH, the milk casein and other proteins reach their isoelectric point forming a gel. There are different types of fermented milk depending on the LAB employed.

The most typical products are obtained after mesophilic fermentation. In this case, the milk fermentation is conducted at 20–30°C producing lactic acid. Some examples of mesophilic fermented beverages are:

- *Cultured buttermilk* is a low-acid fermented milk with an aromatic diacetyl aroma and with a viscous texture. It was originally a by-product of butter manufacture but now is usually produced from milk. This product is traditionally consumed in Germany and Scandinavia as an ingredient in baking production.
- *Nordic fermented milk* is a milk with more viscous consistency and a mild and sour taste. Some examples are the cultured milk called: "tätmjölk” and “Filmjölk” from Sweden. These milk products are often consumed during the meals. Other milk like “lactofil” and “ymer” are centrifuged after fermentation to concentrate the product by removing the whey.

The most typical products obtained by thermophilic fermentation are commented in the following lines. These products are obtained at 42–45°C with lactic acid production.

- *A drinkable yogurt* is a yogurt that has been homogenized to reduce its viscosity to have a liquid texture. It is obtained with a modern well-controlled process using cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Each one has a function, *S. thermophilus* uses essential amino acids produced by *L. bulgaricus* in the first step of the fermentation and produces lactic acid. Lactic acid reduces the pH to a level that is optimum for *L. bulgaricus* development. Meanwhile, *S. thermophiles* are inhibited a pH lower than 4.2, *L. bulgaricus* tolerates well pHs between 3.8 and 3.4. From a nutritional point of view, a yogurt poses lower amount of lactose and higher content of lactic acid and galactose. The total protein content is the same as the original ingredients but due to the action of proteinases, the free amino acid content is higher. The fat content depends on the milk employed (cow, buffalo, sheep, etc.) and also depends on the type of milk used, that is, no-fat, low-fat, or whole-fat milk, but the Codex for fermented milk established a maximum content of fat of 15% (Table 2.2). In addition, certain microorganism synthesizes vitamins so the total content of hydrosoluble vitamins is usually higher in yogurt than in the original milk.

Yogurt is generally well tolerated by lactose-intolerant individuals due to the in vivo release of β-galactosidase of yogurt microbiota. Moreover, conjugated linoleic acid present in milk and with putative atheroprotective properties can be enriched by a LAB that has linoleate isomerase. The proteolytic capacity of LAB can result in a higher quantity of bioactive peptides. These peptides have bioactive properties and have been found in yogurt, sour milk, kefir, and so on. Of important interest, the peptides present in fermented dairy products that have antihypertensive angiotensin-converting enzyme (ACE) inhibitors (Marco et al., 2017).

Yogurt made with cow milk is typically produced in the Mediterranean countries but also in Asia, Africa, and central Europe. There are variations of yogurts made with another kind of milk like "Dahi" in India and "Zabady" in Egypt made of Sheep's milk (Surono and Hosono, 2011). Dahi is an old indigenous fermented milk like yogurt consumed in India. Nahar et al. (2007) published a work in which the protein content of Dahi produced with cow and goat milk has 3.8% and 3.3%, respectively, while the fat content was 4.0% and 3.7%, respectively. It is richer in folate, riboflavin, and niacin than milk and the availability of calcium are also higher than the original milk (Nahar et al., 2007). In the last years, bio-yogurts are gaining acceptability due to the charge of live microorganisms (*L. acidophilus* and *B. bifidum*). Some of them can be labeled as "probiotics" others only as "Contains live and active cultures."

- *Acidophilus milk*. This product is obtained after milk fermentation with *L. acidophilus* during 12–24 h. The milk must be boiled before being incubated with bacteria to eliminate competition and to assure an easy grow of *L. acidophilus*. Afterwards, milk is kept cold until it is consumed. *L. acidophilus* resists the pH of the acid and the bile acids of the intestinal tract. This milk has a strong acid taste so it is predominantly consumed for its known health properties than for its flavor. Acidophilus milk is known to alleviate lactose maldigesting (Onwulata et al., 1989).

- *Bifidus fermented milk*. This product is very similar to acidophilus milk. It is obtained after inoculating the sterilized milk with 10% *L. acidophilus* and *Bifidobacterium*. This milk is used for the treatment of gastrointestinal and liver diseases and flu. Bifidus milk is easy to digest and is used as an infant milk (Kandylis et al., 2016).

Some of the products mentioned before can be called probiotic fermented milk when they contain enough probiotic bacteria in the moment of being consumed. These milk products are made with specific strains of the recognized probiotic bacteria. The most known probiotic bacteria used in milk fermentation are *L. acidophilus*, *L. casei*, and *Bifidobacterium* spp.

2.2.1.2 Fermented Kinds of Milk Obtained From Yeast and LAB

- *Kefir* is a fermented milk beverage with a low percentage of alcohol, obtained using kefir grains. The kefir grains, resemble a cauliflower and are a symbiotic culture of LAB and yeast combined with casein and complex sugars in a polysaccharide matrix (Bourrie et al., 2016). Furthermore, nonlactose fermenting yeast and acetic acid bacteria can be found in kefir grains. Its composition has a proper flavor, which is attributed to a mixture of different substances formed after the fermentation processes (lactic acid, ethanol <2%, carbon dioxide, volatile acids, etc.). It is believed to be originated in North Caucasus Mountains.

  Kefir microbiota composition varies according to the medium employed and also to the type of milk employed. Kefir is produced industrially in many countries in Europe and it is also homemade traditionally in countries such as Turkey, Portugal, and so on. Kefir is rich in water-soluble vitamins, protein, and minerals. Furthermore, the beneficial effects of regular consumption of kefir are associated with improvement in digestion and tolerance to the lactose, antibacterial effect, control of plasma glucose, hypocholesterolaemia effect, antihypertensive effect, antiinflammatory, antioxidant activity, etc. To probe this effects, systematic clinical studies have to be done to better understand the mechanism kefir actuates to prevent such a huge number of diseases (Rosa et al., 2017).
- *Ayran* or *Koumiss* is a salted fermented milk drinkable of low viscosity made of equine or goat milk but also cow milk obtained after a yeast—LAB fermentation. It is traditionally consumed in Turkey, Bulgaria, and Macedonia during the summer and it is usually prepared daily for consumption. Similar products are produced in other countries such as ayrani (Cyprus), dhalle (Albania), and ariani (Greece) (Baschali et al., 2017). This drink has better healthy properties than the original milk as it contains additional peptides and bactericide substances.

- *Tarag* is a traditional naturally fermented drink made of goat milk typically consumed in China. Mongolian community consumed an average of 1–2 L per day per person. As it is usually produced in the houses and not industrially, there is lack of nutrient composition.
- *Kurut* is a fermented yak milk from China. It contains higher LAB and yeast counts than other fermented milk.

2.2.1.3 Fermented Milk Made With Mold and LAB

- *Viili* is an unusual fermented milk made in Finland in which molds are used. This milk is the result of the action of the mesophilic LAB and a surface-growing fungus which forms a velvet surface on the milk. The final milk contains approximately 0.9% of lactic acid and 2.5%–3.9% of fat. It has a mildly acidic flavor and aroma with a thick consistency.

2.2.2 Whey-Based Fermented Milk

Whey is a waste product in the cheese industry. In the last years, great interest has been aroused in the elaboration of fermented beverages with whey to give value to this product. A whey-based beverage can be obtained from milk, whey, and other ingredients (Farah et al., 2017). For example, a kefir done with whey has been developed which contained recognized probiotic bacteria live, and it is expected to have good acceptability in the population (Koutinas, 2016; Marsh et al., 2014). In addition, the supplementation of dairy beverages with whey protein concentrate has a positive effect on the viability of *L. acidophilus* and *Bifidobacterium* during storage. Whey proteins provide a buffering capacity causing a positive effect on probiotics survival (Marco et al., 2017).

2.3 Nondairy Fermented Beverages

Nowadays, new consumer patterns toward healthier and vegetarian diets and higher preference for added-value functional foods have led to an increasing interest in the development of nondairy fermented beverages. These beverages fulfill the requirements of being low in fat, they are a source of bioactive compounds, milk free and, therefore, suitable for some allergic and intolerant consumers.

The high nutritional density of plant foods (carbohydrates, minerals, vitamins, antioxidants, and dietary fiber) makes them excellent options for probiotic beverages production (Santos et al., 2017; Semjonovs et al., 2014). The figures of nondairy milk market as an alternative to dairy milk consumption reflect this new reality since the sales of nondairy milk grew up to 9% in 2015 at the expense of the traditional role model, the dairy milk, whose sales dropped by 7% and it is estimated to continue dropping another 11% by 2020, according to the Mintel market agency (MINTEL, 2016). Particularly, the nondairy probiotic market was estimated to grow up by 15% annually between 2013 and 2018 (Marsh et al., 2014).

Many cultures around the world present culinary practices in which fermentation of plant-based foods is applied to increase their shelf life. These foods are still the dietary and economic livelihood of many populations in undeveloped or developing countries. Therefore, there is a considerably recent research gathering traditional fermented foods from different regions of the world in order to study their safety and nutritional value as well as their processing methods and the possibility of large-scale production for society empowerment (Das et al., 2016; Misihairabgwi and Cheikhyoussef, 2017; Simatende et al., 2015; Tamang et al., 2016). Deep research in this field can promote improvements in the health status of a population strongly dependent on these foods.

For their part, the main interest of developed countries in nondairy fermented foods lies in their potential as functional foods, as they can provide antioxidants compounds and operate as vehicles of probiotic bacteria, both functionalities acting as health developers. As previously mentioned, it is important to keep in mind that a fermented beverage is not a synonym of probiotic beverage. In the following lines, a review of new trends in nondairy fermented beverages is mainly focused on nonalcoholic probiotic beverages, therefore, a distinction will be made when referring to alcoholic beverages as wines and beer.

2.3.1 Microbial Viability in Plant-Based Fermented Foods

Many of the current research are focused on the design and development of new plant-based beverages with functional features. Raw fruit and vegetable materials are harsh environments for microorganism growth due to their acid conditions, high sugar content, presence of nondigestible nutrients, antinutrients, and antigrowth factors, where some phenol compounds are included (Filannino et al., 2014). As mentioned further to guarantee health benefits viable probiotics count should be present in densities of at least 10^6–10^9 viable CFU/day (Bielecka, 2006) (FAO and WHO, 2001), so it is crucial to guarantee bacteria survival during the storage and pass through the upper gastrointestinal tract in order to reach the intestine in enough amounts.

The bacteria most commonly involved in the production of plant-based probiotic beverages belong to the *Lactobacillus* genus—*L. plantarum*, *L. casei*, *L. brevis*, *L. pentose*, *L. delbrueckii*, *L. pontius*, *L. acidophilus*, *L. acetotolerans*, *L. sanfrancisco*, etc. (Gobbetti et al., 2010) and to the *Bifidobacterium* genus—*B. lactis*, *B. bifidum*, *B. breve*, *B. longum*, *B. infantis*, etc. (Semjonovs et al., 2014). This vast group of bacteria species is included in the LAB group as they produce organic acids—mainly lactic acid—from glucose, among other compounds involved in flavor and aroma development. *L. plantarum* is the most commonly used probiotic strain in plant-based fermented beverages as it is able to grow in mediums rich in polyphenols (Shori, 2016). *Bifidobacterium* needs more complex growth factors from the substrate to rise, thus yielding lower growth rates in vegetable juices (Semjonovs et al., 2014), but in combination with *Lactobacillus* strains may increase functional features of the product.

First of all, food matrix obviously can affect cell growth if no enough substrate for bacteria or growth factors is available, or if there is presence of antinutrients or bactericide compounds. Many recent studies are focused on the influence of food matrix on cell growth, viability and sensory properties, and acceptance of the final product (Filannino et al., 2014; Ray et al., 2016). pH may also affect cell growth; values below 3.5 have been found to inhibit *L. plantarum* growth in the development of an apple juice fermented beverage, whereas higher values promoted their rise (Dimitrovski et al., 2015). In this study, more energetic growth at higher initial pH values of the juice was linked to the worst taste score. However, pH decrease and high acidity obtained during fermentation can result in a rough environment for pathogenic microorganisms (Barat and Ozcan, 2017).

Addition of sucrose to cashew apple juice has led to an increase of probiotics viability along the storage time (Pereira et al., 2013). In another case, the addition of sugar and low methylation degree pectin has been found to improve survival of *L. rhamnosus* strain in a fermented beverage of passion fruit under simulated gastrointestinal conditions and after 28 days of refrigerated storage (Santos et al., 2017). Alternatively, in another study, cell growth was negatively correlated with malic acid and sugars concentrations, thereby finding a narrower *L. plantarum* growth in fruits juices compared to that of vegetables (Filannino et al., 2014). Supplementation of vegetable juices with nondigestible carbohydrates as FOS or inulin may enhance bacterial growth and survival if the strains are able to metabolize these complex carbohydrates (Semjonovs et al., 2014; Valero-Cases and Frutos, 2017). The viability of *B. lactis* Bb12 at 4°C storage was enhanced by FOS addition, especially by those of high molecular weight (Semjonovs et al., 2014). NaCl, for their part, can both increase safety and sensory quality of the product and inhibit LAB growth, depending on its concentration in the medium, thus concentration $>5\,\mathrm{g\,L^{-1}}$ has been found to be a nonacidification promoter in cabbage juice (Semjonovs et al., 2014). Therefore, although a higher nutrient medium is essential for LAB growth, toxics accumulation comes along with may result in a lower bacteria count as nutrients increased (Chwastek et al., 2016).

Today, strains combination is a typical solution to improve cell growth in plant-based fermented beverages production (Semjonovs et al., 2014). Chwastek et al. (2016) assessed the effect of the strain employed to produce a red beet fermented beverage supplemented with highbush blueberry osmotic syrup, over the product final concentration of some bioactive compounds as betanin and phenols. The red beet juices with a major number of positive health attributes where those obtained with *L. paracasei* and *L. brevis* strains blended with 10% or lower osmotic syrups. The blending of vegetable juices and/or fruits is supposed to increase their micronutrients value and may improve the palatability and sensory properties of the product. According to a recent in vitro study in which a high number of strains were isolated from sugar water kefir grains, *L. casei* 17U, *Lactobacillus kefiranofaciens* 8U, and *Lactobacillus diolivorans* 1Z showed very suitable features for their use as probiotics due to their antagonism against numerous pathogenic bacteria, to their moderate resistance to artificial gastric conditions, and to their potential ability to adhesion to intestinal cells (Zanirati et al., 2015).

Storage time may adversely affect the cell viability, becoming evident a decrease on viable cells during storage at refrigeration temperatures (Dimitrovski et al., 2015; Filannino et al., 2014) depending also on the matrix and bacteria strain used. However, storage times as many as 32 days have resulted in proper cell densities making sure a possible probiotic effect of the beverage on the human host (Dimitrovski et al., 2015). Microencapsulation of probiotic cells emerges as a tool to enhance bacteria survival during storage and after consumption compared with the beverage with free cells (Dimitrovski et al., 2015; Vatakt and Leenanon, 2017), but more research has to be done in order to minimize its drawbacks as, for example, the lower growth rates compared to that of free cells (Dimitrovski et al., 2015). Furthermore, and to conclude, it can be considered that from the microbial symbiotic interactions, growth of relevant bacteria can be encouraged as well as development of particular flavors and aromas.

It is noteworthy that some of the recent research is focused on fermented beverages obtained from blending of fruit/vegetables/cereals juices with whey, which is a by-product of cheese-making industry. Whey arises as a booster of culture growth due to their high nutrient content leading to greater acceptance of organoleptic properties of the final product. Besides, these high nutritional value beverages might be suitable for lactose intolerant subjects depending on the whey proportion used (Dimitrovski et al., 2015).

2.3.2 Nutritional and Functional Properties of Plant-Based Fermented Beverages

In addition to the viability of probiotic bacteria in the final product, particular interest of recent studies has pointed out to added functional value to fermented beverages made from plant sources. Many researchers are paying attention to the phenolic compounds content and profile due to their well-known antioxidant capacity of many of them and beneficial effects on overall human health (San Miguel-Chávez, 2017). Compounds having antioxidant properties include phenolic compounds and their derivatives, carotenoids and other pigments, vitamins, phospholipids, sterols, amino acids, and peptides. LAB may also enhance nutritional value and quality of fermented beverages by improving bioavailability of minerals, digestibility, and organoleptic properties as color, taste, and aroma (Gupta and Bajaj, 2017; Khezri et al., 2016; Laaksonen et al., 2017)

In general, in fruit and vegetable juices, which are foods rich in phenolic compounds, fermentation may affect their phenolic content, and indeed their antioxidant ability, both positively and negatively. In some cases, fermentation processes may increase phenolic content, which is attributed to bacteria metabolism as they release enzymes during the fermentation process that may convert large phenolic compounds in simpler ones exhibiting higher antioxidant ability according to the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Ekinci et al., 2016; Laaksonen et al., 2017; Sun et al., 2015; Zheng et al., 2014). Lactic acid fermentation of cashew apple juice led to an increase of both antioxidant activity and phenolic content compared to the fresh juice, partly because of the inactivation during the storage of enzymes that degrade phenolic compounds (Pereira et al., 2013). Furthermore, malolactic fermentation of apple juice resulted in a significant increase in both quercetin and phloretin compounds, the latter possibly influencing bitterness of the fermented juice (Laaksonen et al., 2017). In a strawberry beverage obtained by gluconic fermentation an increase by 36% on gallic acid during fermentation was attributable to the esterase tannase enzyme produced by certain microbes; likewise, hydroxycinnamic acids and flavonols also have been found to increase during fermentation (Álvarez-Fernández et al., 2014). Sun et al. (2015) study showed enhancement of antioxidant potential of Kombucha tea when blended with wheatgrass juice in 1:1 ratio. Low pH due to fermentation processes can improve stability of anthocyanins, which can be affected by the presence of metal ions and high temperatures (Sun et al., 2015). However, large phenolic contents can inhibit culture growth due to the antimicrobial activity of many of them (Kazakos et al., 2016).

On the contrary, a significant decrease or no changes in total phenolic content, as well as in antioxidant activity, were found by other authors after fermentation in different kefir-like beverages from carrot, fennel, strawberry, onion, tomato, and melon juices, in shalgam juice—which is made from black carrot—and in juices of potato and red beet with blueberry syrup (Ekinci et al., 2016; Kim et al., 2012).

Blending of fruit or vegetable juices with milk or whey is a useful tool to better increase microorganisms' viability, nutritional value, antioxidant capability, and even sensory properties of the final beverage (Barat and Ozcan, 2017; Chilana et al., 2015). Pomegranate and carambola, a native Asia tropical fruit, was found to be an unsuitable medium for bacteria survival due to the presence of antimicrobial compounds (Kazakos et al., 2016). Moreover, whey addition could also result in a worse acceptance of sensory properties by the consumer (Maldonado et al., 2017).

2.3.3 Novel and Traditional Fermented Plant Beverages

In the subsequent sections, a brief description of the novel fermented brews with an interest in the latest research will be done. Particular attention will be paid in probiotic fermented beverages.

2.3.3.1 Nonalcoholic Fermented Beverages Made From Cereals and Legumes

Cereals and legumes are suitable foods for their use as substrate for probiotics growth as they are rich in carbohydrates, proteins, fiber, vitamins, and minerals. They have been traditionally employed in different cultures from Near East, Africa, and Asia, where they stand on the basis of the diet, to produce fermented brews rich in nutrients, nutrients, including prebiotics, that is, nondigestible carbohydrates. Nowadays, meeting consumer demands toward healthier food options have turned out to many research highlighting the probiotic potential of traditional and novel fermented beverages from cereals and legumes. Moreover, the capability of legumes traditionally vilified as bambara groundnut to produce probiotic nutrient-enriched beverages its being explored (Murevanhema and Jideani, 2013).

Cereals used as a substrate of fermentation include wheat, barley, oats, sorghum, rice, maize, rye, and millet, among others (Bhadekar and Parhi, 2016; Freire et al., 2017; Marsh et al., 2014). The development of cereal-based fermented brews with good organoleptic properties is one of the biggest challenges of the cereals industry (Salmerón, 2017). Operational control of fermentation process—inoculum type, size and timing, pH, temperature, environmental stress conditions—may result in special aromas and palatable taste, but its dependency on the substrate is especially relevant (Nsogning Dongmo et al., 2016;

Peyer et al., 2016; Salmerón, 2017). *L. plantarum* is stated to develop pleasant flavors (Peyer et al., 2016).

From the safety point, cereals are prone to spoilage by fungi which also represents a health risk to consumer due to mycotoxins production. In this sense, certain LAB have been proven to have antifungal potential attributed to some fermentation metabolites such as organic acids, CO_2, ethanol, and hydrogen peroxide among others (Crowley et al., 2013). This approach has been successful in the development of a new oat-based beverage obtained by fermentation with *L. plantarum* UFG 121 which resulted in one of the strongest antifungal activities among 88 *L. plantarum* strains (Russo et al., 2017). Table 2.3 summarizes recent research on probiotic beverages made from cereals and legumes. Identification of raw material, microbial inoculum, conditions of incubation, and viability of bacteria at storage, if available, are shown.

Other traditional cereal-based beverages drawing interest in the latest research include the following.

- *Boza*, a traditional brew consumed in Bulgaria, Turkey, and Southern-Eastern Europe, is made from cereal mixed—mainly wheat, rice, millet, and maize—with sugar (Peyer et al., 2016; Prado et al., 2008). This slightly alcoholic brew (1.5%) is obtained by bacteria and yeast fermentation. Boza presents a high diversity of species from the genus *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, *Saccharomyces*, *Enterococcus*, *Weisella*, *Candida*, *Acremonium*, *Aspergillus*, *Penicillium*, *Torulaspora*, *Fusarium*, *Galactomyces*, and *Geotrichum* has been isolated from this popular beverage (Altay et al., 2013; Heperkan et al., 2014; Prado et al., 2008).
- *Amazake* is a Japanese sweet beverage made from rice. It is the low-alcohol precursor of sake obtained during the first stage of fermentation by the fungi *Aspergillus oryzae* (Marsh et al., 2014; McKay et al., 2010). This beverage seems to have a strong antioxidant potential.
- *Chyang*, original from the North-Eastern of India, is a mild-alcoholic beverage made from barley or finger millet—also called ragi—thoughtful as a nutrient-dense beverage as remedy against common illnesses and for woman to postnatal recovering (Das et al., 2016; Ray et al., 2016). It is produced using a mixed culture called *Marcha* which includes yeasts species from the genus *Saccharomyces*, *Candida*, *Saccharomycopsis*, and *Pichia* and LAB and molds from *Rhizopus* and *Mucor* genus (Kitamura et al., 2016; Ray et al., 2016).
- *Bushera* is a Ugandan beverage mainly obtained from shorgum but also from millet. At the moment of consumption, fermentation is still active. Strains from the species of *L. fermentum*, *L. plantarum*, *L. paracasei* subsp *paracasei,* and *Weisella confuse* were isolated from Bushera.

Table 2.3 Briefly Description of Developed Fermented Beverages Made From Cereals and Legumes

Functional Food	Raw Material	Microorganism	Incubation	Viability at Storage	Other Functional	Reference
Fermented oat flour beverage	Oat flour and honey	*L. plantarum*	*T*=37°C for 48 h	10^{14} CFU/mL after 4 weeks at 4°C	–	Gupta and Bajaj (2017)
Fermented beverage from maize and rice	**1)** Maize + FOS **2)** Maize-rice blend + FOS	*L. plantarum* + *Torulaspora delbrueckii* + *L. acidophilus*	*T*=37°C <36 h	10^{7} CFU/mL after 28 days at 4°C	–	Freire et al. (2017)
Barley malt wort	Barley malt	*L. plantarum* L.p	72 h	–	–	Nsogning Dongmo et al. (2016)
Purple glutinous rice beverage	Purple glutinous rice	*Lactobacillus pentosus*	–	*Encapsulated cells*: 8.77 log CFU/mL *Free cells*: 8.36 log CFU/mL after 35 days at 4°C	–	Vatakt and Leenanon (2017)
Fermented soymilk and camu-camu beverage	Soymilk and camu-camu	**1)** *L. plantarum* **2)** *L. helveticus*	*T*=37°C <72 h	–	Antihyperglycemic and antihypertensive properties	Fujita et al. (2017)

Oat-based synbiotic beverage	Oat	*Rhizopus oryzae* + *L. acidophilus*	*R. oryzae*: T=25°C for 3 days *L. acidophilus*: T=37°C for 12 h	~ 8.5 log CFU/mL at the end of fermentation	Source of β-glucan (anticarcinogenic, action against pathogenic bacteria, insulin and cholesterol regulator)	Gao et al. (2012)
Barley malt fermented beverage	Barley malt wort	*Weissella cibaria*	T=30°C for 72 h	10^7 CFU/mL after 72 h	–	Zannini et al. (2013)
Cereals-based fermented beverages	**1)** Malt extract **2)** Rice **3)** Khorasan wheat **4)** Wheat flour	**A)** *S. cerevisiae* var. *boulardii*; **B)** *Kluyveromyces lactis* **C)** *Saccharomyces pastorianus* **D)** *Kazachstania exigua*	T=4°C for 24 h	7 log CFU/mL in rice drink	–	Bevilacqua et al. (2015)
Fermented potato juice	Potato	*L. casei*	T=37°C for 72 h	5–9 log CFU/mL after 4 weeks at 4°C	–	Kim et al. (2012)
Fermented soymilk beverage with apple juice	Soy milk and apple juice	*L. acidophilus*	T=37°C for 24 h	~9 log CFU/mL after 21 days at 4°C	–	Içier et al. (2015)

2.3.3.2 Alcoholic Fermented Beverages Made From Cereals and Legumes

Not less important are the popular alcoholic cereal-based beverages, being beer the most worldwide known drink of this group. The term “beer” comprises a group of beverages in which alcoholic fermentation of sugars present in malted cereals (barley, wheat, rice, etc.) occurs. They usually present an alcoholic content between 3% and 12%. The most common beer is that made from barley or wheat but that of rice is quite usual in Asia (Hossain and Kabir, 2016). In addition, there is an increasing interest of sorghum malt for substrate in drinks industry as it is comparable to barley malt. The latter is associated with important health benefits such as anticancer properties and protection against pathogenic bacteria that colonize the gut due to the presence of bioactive compounds (Das et al., 2016). Sake, which is a popular non-distilled rice-based brew original from Japan, is obtained in a process similar to that of the beer and containing around 15%–20% alcohol. It is produced by symbiotic interaction of *koji* mold—*A. oryzae*—, which objective is saccharified the starch of steamed rice, yeast and LAB—*S. cerevisiae, L. plantarum*, and *L. leichmanni*—, in a multiple parallel fermentation where saccharification and alcoholic fermentation take place simultaneously (Kitamura et al., 2016; McKay et al., 2010). A strong enzymatic activity of *koji* mold is requested in the manufacturing of sake. While *S. cerevisiae* is employed for the development of alcoholic fermentation, LAB are added to prevent growth of undesirable microorganisms (Kitamura et al., 2016; McKay et al., 2010).

Consumption of Beer and Sports Recovery

Alcoholic beverages have been consumed for thousands of years, attracting great human interest for social events and personal enjoyment. Although excessive consumption of alcoholic beverages is commonly regarded to be detrimental to health, there have long been debated to confer health benefits in low or low-moderate amounts (Haseeb et al., 2017). Beer with low-alcohol content has an isotonic osmolarity, as well as other types of sports drinks. The composition of beer, with alcohol and without alcohol, offers the opportunity to be used as an option among the beverages used by athletes for the replacement of nutrient loss after finishing physical activity. This is due to its high-water content (92%) with a moderate energy input (30–40 kcal), to its content in all the essential amino acids and some nonessential amino acids, as well as in phosphorus, magnesium (Nielsen and Lukaski, 2006), and potassium (Maughan et al., 2004). These salts favor the hydro-electrolyte replacement with the intake of liquids to replace the dehydration caused by sweating during exercise. Moreover, the moderate intake of beer can be used as a rehydrating drink in hot environments after performing physical activity (Jimenez-Pavón et al., 2015).

The consumption of beer in athletes is not one of the most studied issues today, however, it is a subject of great interest. The effects of alcohol could have an influence on aspects related to the behavior of individuals and anxiety parameters, so it is interesting to be able to analyze the incidence of beer consumption and set up a relationship with trait anxiety. Furthermore, as for the appetite, the consumption of alcoholic beverages, can induce an increase of this one (Yeomans and Phillips, 2002), which is diminished immediately after performing an extenuate physical activity, due to the fatigue that this entails. In this sense, the consumption of alcoholic snacks stimulates the appetite (Westerterp-Plantenga and Verwegen, 1999).

Alcohol-free beer is considered an important antipathogenic, and antioxidant source with antiinflammatory properties (Scherr et al., 2012), which can also be very useful in sports recovery. Furthermore, the consumption of beer without alcohol seems to have a satiating effect on subsequent meals (Yeomans and Phillips, 2002), a fact that could be very useful especially in order to control dinners and make lighter intakes to be able to have a light digestion at night not affecting the hours of sleep. The consumption of beer without alcohol is recommended due to its content of hops and the consequent sedative action, which would be an aid for night-time sleep.

In terms of body composition, hip-waist ratio (Dallongeville et al., 1998) could be modified when there is alcohol consumption. For this reason, athletes must control the total energy intake based on total energy expenditure associated with different training and daily physical activities (Ainsworth et al., 2011) for supporting their body composition stable and avoiding increasing their fat mass. A systematic review (Barnes, 2014) showed no impact of beer consumption on athletes' performance, but it is quite the opposite, as due to its properties and diuretic it could contribute to an alteration of body mass through the incidence of beer on lipid metabolism.

2.3.4 Fermented Beverages Made From Vegetables and Fruits

Some of the latest research deals with the development of novel fermented beverages from fruits, vegetables, or even mixing of both in order to improve nutritional and health properties of the final product. Vegetables and fruits exhibit antihypertensive, antidiabetic, antioxidant, and cholesterol-lowering properties because of the presence of bioactive compounds (Simsek et al., 2014). Moreover, in vitro antiproliferative effects of shalgam juice have been demonstrated (Ekinci et al., 2016).

Compared to cereals and legumes-fermented beverages, those of fruits and vegetables develop more flavorful properties. Fruit-based fermented beverages show a more marked acidic taste and a thicker consistency due to the higher bacteria biomass compared to fresh juice (Ekinci et al., 2016; De Souza Neves Ellendersen et al., 2012). A short description of innovative probiotic beverages made from fruits and vegetables is drawn in Table 2.4. Bearing in mind that fruits can present autochthones and/or undesirable microorganisms from origin, and that they can easily pass into the juice during processing, new disinfection methods as the use of high hydrostatic pressures, have emerged as an alternative to pasteurization to complete inactivation of juice microbiota causing minimal effects on nutritional and sensory properties of juice (Zheng et al., 2014).

Other traditional plant-based beverages with research interest in the last decade are shown below:

- *Kombucha* was originated in China and is commonly called tea fungus. It is a very popular beverage consumed worldwide with an increasing market share due to many health claims such as detoxificant, antioxidant, increase longevity, and enhance gastrointestinal status (Marsh et al., 2014). However, more research has to be done to provide harder evidence about its health properties. Nowadays is produced from sweetened black tea and a mixed culture known as SCOBY including acetic acid bacteria and yeasts species from the genus *Acetobacter*, *Gluconobacter*, *Saccharomyces*, *Torulopsis*, *Pichia*, *Brettanomyces*, and *Zygosaccharomyces*, among others. Due to the presence of these microorganisms, sugar is metabolized into ethanol which is then oxidized to acetic acid, so it exhibits a slightly alcoholic content of 0.5%. Because of its high acidic content, pH ~2, it has an acidic taste similar to that of vinegar (Marsh et al., 2014). Kombucha fermentation using lemon balm resulted in a higher antioxidant activity compared to the traditional kombucha.
- *Hardaliye* is a homemade Turkish nonalcoholic beverage made from red grapes where black mustard seeds are added to increase palatability (Bhadekar and Parhi, 2016). Regarded as a probiotic beverage, different species of *Lactobacillus* as *L. paracasei*, *L. casei*, *L. pontis*, *L. brevis*, *L. acetolerans*, *L. sanfrancisco*, and *L. vaccinostercus* has been reported to produce hardaliye. Amoutzopoulos et al. found a decrease in some oxidative stress biomarkers status after daily supplementation of hardaliye during a period of 40 days.

The most well-known alcoholic plant-based beverage is wine which is made from grape juice followed by an alcoholic fermentation of sugars mainly by yeasts of *Saccharomyces* genus. Vinification of different fruit juices is also a trend in interest in the recent research as shown in two studies about pomegranate and raspberry wines.

2.3.4.1 Consumption of Wine and Sports Practice

As in the case of beer consumption and exercise, scientific contributions observed that a moderate daily consumption of red wine has

Table 2.4 Briefly Description of Developed Fermented Beverages Made From Vegetables and Fruits

Functional Food	Raw Material	Microorganism	Incubation	Viability at Storage	Other Functional	Reference
Probiotic cashew apple juice	Cashew apple	*L. casei*	–	10^8 CFU/mL after 28 days at 4°C	Higher ascorbic acid content after 42 days (290 mg/L)	Pereira et al. (2013)
Vegetable probiotic beverage	Beetroot, carrot, celery and honey	**1)** *L. acidophilus* **2)** *L. casei* **3)** *Saccharomyces. boulardii*	8–9 h	1) & 2) 7.7–8.5 log CFU/mL 3) 7–7.5 log CFU/mL	–	Profir and Vizireanu (2013)
Probiotic pumpkin juice-based beverage	Pumpkin	*Lactobacillus reuteri*	T=37°C for 48 h	7 log CFU/mL after 4 weeks	–	Semjonovs et al. (2014)
Fermented litchi juice	Litchi	*L. casei*	T=30°C for 18 h	10^8 CFU/mL after 4 weeks at 4°C	–	Zheng et al. (2014)
Probiotic apple beverage	Fuji and Gala apples	**1)** *L. casei* **2)** *L. acidophilus*	T=37°C for 10 h	8.5 log CFU/mL after 28 days at 7°C	–	De Souza et al. (2012)
Fermented beverages of vegetable juices	**1)** Carrot **2)** Fennel **3)** Melon **4)** Onion **5)** Tomato **6)** Strawberry	*Lactobacillus* + *Lactococcus* + *Leuconostoc* + *Saccharomyces* (water kefir microorganisms)	1st T=25°C for 72 h 2nd T=25°C for 48 h	8–9 log CFU/mL at the end of fermentation	–	Corona et al. (2016)

Continued

Table 2.4 Briefly Description of Developed Fermented Beverages Made From Vegetables and Fruits—cont'd

Functional Food	Raw Material	Microorganism	Incubation	Viability at Storage	Other Functional	Reference
Fermented moringa leaves and beetroot beverage	Moringa leaves and beetroot	*L. plantarum* and *Enterococcus hirae*	*T*=37°C for 48 h	10^7 CFU/mL after 30 days at 4°C	–	Vanajakshi et al. (2015)
Fermented carrot and orange juice	Carrot and orange juices and artichoke inulin	*L. plantarum*	*T*=37°C for 24 h	9.2 log CFU/mL after 30 days at 4°C	–	Valero-Cases and Frutos (2017)
Fermented mango and guava juices	Mango and guava juices	*L. casei*+*Streptococcus thermophillus*+*L. bulgaricus*	*T*=37°C for 72 h	10^8 CFU/mL after 24 h	–	Maldonado et al. (2017)
Wheatgrass-blended kombucha beverage	Sweetened black tea and wheatgrass juice	*Gluconacetobacter rhaeticus*+*Gluconobacter roseus*+*Dekkera bruxellensis*	*T*=29°C for 12 h	–	Higher antioxidant activity	Sun et al. (2015)
Cabbage juice-based fermented functional symbiotic beverage	Cabbage juice	Bifidobacterium lactis	*T*=37°C for 48 h	7 log CFU/mL after 2 weeks at 4°C	–	Semjonovs et al. (2014)

been proposed to contribute to improving some health parameters. In fact, epidemiological studies from diverse populations have revealed that moderate amounts of wine can led to a 20%–30% reduction in all-cause mortality (above all cardiovascular illness) when compared with individuals who abstain or who drink alcohol to excess (Guilford and Pezzuto, 2011). Red wine has been shown to increase plasma antioxidant capacity, suppressing reactive oxygen species generation, increasing serum oxygen radical absorbance capacity, and decreasing oxidative DNA damage or chronic inflammation (is a critical component in many human diseases and conditions, including obesity, cardiovascular diseases, neurodegenerative diseases, diabetes, aging, and some types of cancer), and are capable of modulating immune responses (Droste et al., 2014).

There are some exercise benefits reported by the literature related to wine consumption. Consumption of red wine has been associated significantly with a decreasing systolic blood pressure. In connection with the influence of moderate daily wine consumption on body composition, alcohol has a high-energy density ($7.1\,\text{kcal}\,\text{g}^{-1}$) similar to fat ($9.0\,\text{kcal}\,\text{g}^{-1}$), so it is commonly assumed that alcohol could affect negatively at body composition, increasing the body weight and fat mass (Cordain et al., 1997). For the one hand, scientist suggested that moderate or regular alcohol consumption favors the development of obesity via its lipid oxidizing suppressive effects or by its failure to induce a compensatory reduction in lipid and nonalcoholic energy intake. On the other hand, some epidemiological reports show either no association or an inverse association between alcohol consumption and body weight gain. Consequently, more scientific evidence relating to long-term metabolic, caloric and body weight changes is necessary, so the conclusions drawn can be misleading or, at least, treated with caution. Additionally, drinking a moderate quantity of alcohol (two glasses of wine per day), for people who include in their lifestyle enough physical exercise have not influence in their body weight, body composition, resting metabolism, or substrate utilization in any way which may promote the development of obesity.

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3

FERMENTED BEVERAGES PRODUCED BY MIXED CULTURES, PURE CULTURES, AND DEFINED COCULTURES

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3.1 Historical Development in Beverage Production

Fermented beverages have been consumed for several thousand years all over the world, and for most of that time the fermenting microorganisms were not known at all. The traditional fermentation processes occurred by spontaneous, mixed culture fermentation of carbohydrate-rich substrates. The achieved fermentation products are strongly dependent on the substrate, the naturally occurring microorganisms involved in the fermentation and the fermentation conditions, such as temperature, pH, and oxygen availability. Fermented beverages exhibit several beneficial properties like attractive flavor, good digestibility, increased nutritional value, and a reduction of pathogenic microorganisms (Bourdichon et al., 2012). The latter led to the utilization of beer as a secure beverage, as compared to water in medieval times. The explanation for this started to develop with the discovery of microorganisms by Antonie van Leeuwenhoek in 1683. Afterwards, it took nearly 200 years until Louis Pasteur discovered in 1857 that yeast is responsible for alcoholic fermentations. This knowledge was applied by Max Emil Julius Delbrück, who developed the first pure yeast culture in 1895. The sterile fermentation of pure cultures offers the possibility of developing well-controlled processes as they are applied in modern beer brewing processes. While pure culture processes lead to reproducible fermentation products with constant flavor

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quality, predictable shelf life, and consistent texture or "mouthfeel," especially in beverage production, there still exist many fermentation processes that rely on spontaneous mixed cultures or cocultures, such as wine or Belgian beers. The amount of different microorganisms in mixed fermentations offers the advantage of producing many different flavor-active substances, resulting in a huge variety of flavor profiles. Especially in wine production, different flavor profiles are favored and the quality of fermentation products alters between location and year. These differences can be minimized when the fermentation is inoculated with a high concentration of a single microorganism for example, *Saccharomyces cerevisiae*. The disadvantage of this strategy is the reduction of flavor complexity. A compromise between a controllable process and a rich flavor profile can be the application of the controlled coculture process. In this case, inoculation is conducted with a mixture of selected microorganisms (yeasts and bacteria). Undesired microorganisms are not able to proliferate and simultaneously many flavor active substances are produced. Furthermore, alcohol content of the fermentation product can be influenced by the applied microorganisms. An actual summary of microorganisms applied in food industry is provided by The European Food and Feed Cultures Association—EFFCA (http://www.effca.org/).

3.2 Fermented Beverages

In the following section, we provide an overview about fermented beverages. The collection was limited to fermented beverages in the liquid phase. Fermentations of solid substrates, such as tea leaves, coffee or cacao beans, and vegetables are not included. Information about these processes can be found in Bader et al. (2018).

3.2.1 Alcoholic Beverages

For thousands of years, alcoholic beverages have been continuously consumed by humans worldwide. The production of these beverages was traditionally conducted by spontaneous mixed fermentation without any knowledge of the participating microorganisms. Even today, many traditional alcoholic beverages are produced without specific knowledge of the participating microorganisms, while others are fermented under sterile conditions using pure cultures. The best known fermentation product derived from a mixed culture of many bacteria and yeast strains in a non-sterile mixed culture is wine. Barley-based beer is one of the best known fermentation products achieved by sterile fermentation with single yeast. The worldwide average consumption of pure ethanol among people older than 15 years is 6.2 L per year but there are strong variations between countries (WHO, 2017). This amount of alcohol is consumed in the form of beer and beer-like beverages (based on barley, sorghum, or corn), wine (based on grapes, other fruits, or rize), spirits (based on grains, potatoes, or fruits), and other fermented beverages like mead. Several examples are presented in Table 3.1.

In most of the presented examples of alcoholic beverages, more than one microorganism is active during the fermentation process. Beer is an example of a pure culture fermentation process, while the other products are produced by mixed cultures or cocultures. The required reduction of microorganisms on the fermentation substrate during beer production is achieved by boiling the wort. Afterwards, the substrate is not sterile, but inoculation with a high concentration of yeast followed by the production of ethanol, CO_2, and the decreasing pH value helps to prevent proliferation of undesired microorganisms.

Wine and many other of the examples presented in Table 3.1 are produced by spontaneous fermentation of many different microorganisms. Graça et al. (2015) could identify more than 200 yeast strains on 25 apple samples, which indicate the complexity of the fermentation when wine is produced with these apples. Furthermore, it shows that the presented microorganisms in Table 3.1 are only a small number of possible microorganisms. Wide variations are possible at the beginning of the fermentation process. During the fermentation process, a selective pressure develops, leading to the predominance of small groups of anaerobic, ethanol tolerant organisms. One group of these organisms is *Saccharomyces* spp., but many others are able to contribute to the flavor of the beverage. Belda et al. (2016) reported about a strong influence of non-*Saccharomyces* strains on the flavor production. Also, the interactions between participating microorganisms influence flavor production (Gobbi et al., 2013). However, the flavor is not the only aspect of the beverage influenced by microorganisms participating in the fermentation. Also, the final concentration of ethanol in the product is influenced by the type of yeast or bacterium used.

To ensure a stable and reproducible fermentation process, pure yeast strains have been applied as starting cultures for the production of wine from grapes but the resulting wines produced by this method were comparably poor in flavor (Capece et al., 2013). Based on these results, pre-cultures consisting of different selected strains had been developed to produce a fermentation product with stable and reproducible properties without a reduction in the concentration of key flavor substances (Terrell et al., 2015). Berbegal et al. (2016) could show that inoculation with *S. cerevisiae* and *Lactobacillus plantarum* led to a wine with very high quality, due to the improved malolactic fermentation.

Mixed cultures and cocultures are also applied in beer production. Especially, the production of the Belgian Lambique (Lambic) beers is

Table 3.1 Examples of Worldwide Produced Alcoholic Beverages Including the Utilized Substrates and Microorganisms

Product Name	Substrate	Participating Microorganisms	Typically Produced and Consumed in
Arrack	Coconut	Yeasts, acetic acid bacteria	Sri Lanka, India, Philippines
Beer	Barley, wheat	*Saccharomyces* sp.	Worldwide
Berliner Weisse	Barley, wheat	*Saccharomyces cerevisiae, Lactobacillus* sp., *Brettanomyces bruxellensis*	Germany
Burukutu	Sorghum	*Saccharomyces* sp., *Leuconostoc* sp., *Candida* sp., *Acetobacter* sp.	Nigeria, Benin, Ghana
Busaa	Corn	*Saccharomyces cerevisiae, Lactobacillus* sp., *Penicillium damnosus*	Nigeria, Ghana
Bushera	Sorghum	*Lactobacillus* sp., *Weissella confusa*	Uganda
Cider, apple wine	Apples	*Kloeckera apiculata* and *Metschnikowia pulcherrima*, *Saccharomyces* sp., *Brettanomyces* sp., *Lactobacillus* sp.	France, Germany, Great Britain, United States
Doro	Sorghum	Yeast and bacteria	Zimbabwe
Gose	Barley, wheat	*Saccharomyces* sp., *Lactobacillus* sp.	Germany
Grappa	Grape pomace		Italy
Lambic-Bier	Wheat	*Kluyveromyces apiculata, Saccharomyces* sp., *Brettanomyces* sp., *Pediococcus* sp., *Enterobacteria* sp.	Belgium
Kaffir-Bier	Corn	Yeasts and lactic acid bacteria	South Africa
Khaomak	Rice	*Rhizopus sp.*, *Mucor* sp., *Saccharomyces* sp., *Hansenula* sp.	Thailand
Korn	Grains		Germany
Mahewu	Corn or wheat	*Streptococcus lactis, Lactobacillus plantarum*	South Africa
Mead	Honey	*Saccharomyces cerevisiae, Pichia* sp.	Scandinavia
Merissa	Sorghum	*Lactobacillus* sp., acetic acid bacteria, *Saccharomyces cerevisiae*	Sudan
Pito	Corn, sorghum	*Geotrichum candidum, Lactobacillus* sp., *Candida* sp.	Nigeria, Ghana
Raki	Grapes	*Saccharomyces cerevisiae, Lactobacillus* sp., acetic acid bacteria	Turkey
Rum	Sugarcane	*Saccharomyces cerevisiae, Candida* sp., *Pichia* sp., *Hansenula, Lactobacillus* sp., *Leuconostoc* sp.	Worldwide

Sake	Rice	*Saccharomyces sake, Lactobacillus* sp.	Japan
Seketeh	Corn	*Saccharomyces* sp., *Lactobacillus* sp., *Bacillus subtilis, Aspergillus* sp., *Mucor rouxii*	Nigeria
Tapai pulut	Rice	*Hansenula* sp., *Chlamydomucor* sp., *Endomycopsis* sp.	Malaysia
Tequila/Mescal	Agave	*Saccharomyces cerevisiae, Kluyveromyces marxianus, Zymomonas mobilis*	Mexico
Tupay	Rice	*Saccharomyces* sp., *Mucor* sp., *Rhizopus* sp., *Aspergillus* sp., *Leuconostoc* sp., *Lactobacillus plantarum*	Philippines
Togowa	Sorghum	*Lactobacillus* sp., *Pediococcus* sp., *Weissella confuse, Issatchenkia orientalis, Candida* sp.	Tanzania
Vodka	Grains or potatoes		Russia, Poland
Wine	Grapes, other fruits or rize	*Saccharomyces* sp., *Kluyveromces* sp., *Brettanomyces* sp., acetic acid bacteria, lactic acid bacteria	Europe, Australia, Africa, United States, Asia

Due to local variations alterations of substrates and microorganisms may occur.

dependent on many different microorganisms. These mixed fermentations are traditionally conducted in open fermentation vessels and the fermentation process is strongly influenced by the microbial environment in the brewery. The different compositions of the mixed culture in different breweries offer the advantage of very different flavor formation that is typical of the immediate environment. Problems arise when the brewery has to move to a different building. In this case, the microbial environment changes, and the beer changes its flavor. A transfer of the process with consistent product quality is difficult or even impossible. To overcome this problem, a controlled coculture fermentation with selected strains must be developed.

3.2.2 Beverages With Reduced Alcohol Content

Nonalcoholic beverages (NAB) and beverages with low alcohol content (BLA) are gaining increasing interest of consumers, due to the increasing awareness in well-being and health. For examples, alcohol-free beer is well known, fermented lemonades are available, and there is also alcohol-free wine. Of course, traditional alcohol-free fermented beverages, such as kombucha or kefir have been produced for many years. In many cases, fermented beverages are produced in small scale with traditional recipes. Increasing mainstream interest in these beverages has resulted in the first industrially produced, alcohol-free fermented beverages.

In the following text, a discussion of fermented beverages with no alcohol or low alcohol content is presented. Due to the various legislation of "alcohol-free beverages" in different countries, there will be no differentiation between alcohol-free and low-alcohol containing beverages in the next sections (Table 3.2).

The NAB discussed here offer a good alternative to non-fermented beverages. They exhibit good storage capabilities (i.e., "shelf life") and, in many cases, health benefits are gained from the consumption of these fermented beverages. Although there are many reports and scientific investigations regarding the positive effects resulting from the intake of these fermented beverages (Todorov et al., 2008; Borresen et al., 2012), there is no health claim for probiotic microorganisms in the European Union as of now.

Besides the aforementioned alcohol-free beverages, there is an increasing demand for alcohol-free beverages that exhibit comparable sensory properties as the alcohol containing beverages. This demand results, in part due to the increasing health consciousness of many consumers, for example wine, beer, and sparkling wine. As of today, acceptance of alcohol-free versions of these beverages is quite low, because in many cases they do not taste similar enough to the alcohol containing version. In many cases, the alcohol-free version is

Table 3.2 Summary of Fermented Alcohol-Free or Low Alcohol Beverages

Beverage	Substrate	Microorganisms	References
Ayran	Milk	*Lactobacillus* sp., *Streptococcus* sp.	
Bouza/Boza	Wheat, Sorghum, Corn	*Saccharomyces cerevisiae, Lactobacillus* sp., *Leuconostoc* sp.	
Hardaliye	Grapes, mustard seeds, sour cherry leaf	*Lactobacillus* sp.	Gucer et al. (2009)
Kombucha	Tea	*Gluconobacter* sp., Lactic acid bacteria, *Saccharomyces* sp., *Candida* sp.	Jayabalan et al. (2014)
Kvass	Rye or barley in form of malt or baked bread	Lactic acid bacteria, *Saccharomyces cerevisiae*	Basinskiene et al. (2016)
Milk Kefir	Milk	*Lactobacillus* sp., *Leuconostoc* sp., *Acetobacter* sp., *Kluyveromyces* sp., *Saccharomyces* sp., *Candida* sp.	Prado et al. (2015)
Shalgam/Salgam	Purple carrot	Lactic acid bacteria	
Sima	Lemon		

Presented microorganisms are the mainly prevalent strains. Variations may occur due to different origins.

much sweeter and lacks many aromatic fermentation products. Many alcohol-free beers exhibit a worthy off-flavor (Missbach et al., 2017). There are different biological and physical methods for the production of NAB or beverages with reduced alcohol content. Advantages and disadvantages of the different methods are discussed in the following.

One biological procedure for reduction of the amount of produced ethanol is reduction of fermentation time. In this arrested or limited fermentation processes, the microbial conversion of the substrate is stopped shortly after inoculation with the yeast. This results in a strong wort flavor and a lack of desired flavor substances (e.g., aldehydes and aromatic compounds). Furthermore, there is a high concentration of fermentable sugars in the product and the pH value is comparably high. Both of these phenomena may result in decreased microbial stability of the final beverage.

Other bio-based processes include the application of yeast strains that produce relatively small quantities of ethanol. For example, maltose negative yeast strains can be applied for the production of light beer because the main sugar in beer wort is maltose. If this sugar cannot be fermented, then only low amounts of ethanol are produced. The partial fermentation of sugars means a high intake of sugar for the consumer, because common beer wort contains approximately 100 g/L of sugars. This high sugar consumption is in contrast to the recommendation of the World Health Organization (WHO) of a daily sugar intake of 50 g or even 25 g (World Health Organization, 2015). Alternatively, the utilization of genetically modified organisms (GMOs) can lead to reduced alcohol formation. Nevoigt et al. (2002) published results about an engineered *Saccharomyces* strain that fermented available sugars similar to the wild-type strain, but produced reduced amounts of ethanol. Also, in wine production there are reports about the possibility of reducing the alcohol content through the use of GMOs. Cuello et al. (2017) showed that ethanol production could be decreased during wine fermentation without affecting the aroma formation by using a genetically modified *S. cerevisiae* strain. In this work, ethanol production was decreased by 15%–18%. Application of GMOs might be a possibility for a reduction of alcohol content in beer or wine once consumers are willing to accept GMOs in food production. Especially in the European Union, the acceptance of GMOs in food production is still very low. Therefore, other possibilities are required to reduce the ethanol content of beverages. Non-*Saccharomyces* yeast strains are a good alternative for the production of low-ethanol wines. Contreras et al. (2014) evaluated 50 of these strains for wine production and developed a sequential inoculation process. They achieved an ethanol reduction of up to 1.6%(v/v) by this method with good aroma profiles in Shiraz wine.

As discussed above, with biological methods, only a small reduction of ethanol content is possible when the aroma profile and sugar content of the final, low-alcohol beverage should be comparable to the original product. To overcome these disadvantages, physical methods of alcohol reduction in beverages can be applied. These methods include evaporation, distillation, dialysis, reverse osmosis (RO), and pervaporation (PV) (Brányik et al., 2012; Takács et al., 2007).

Evaporation: Due to the relatively low boiling point of ethanol (78°C), it can be removed from beverages by evaporation. However, there are several disadvantages of this process. The high temperatures cause chemical reactions, which lead to an altered flavor of the beverage. Furthermore, desired aroma-active volatile compounds are removed from the beverage. Also, evaporation requires high amounts of energy. Due to these disadvantages, this strategy is not used for alcohol reduction in beverages. Using vacuum evaporation (VE) reduces the thermal effect. This method is used for the production of alcohol-free beer on an industrial scale.

Distillation: Compared to evaporation, a fractionated removal of ethanol is possible with this process. Desired compounds (e.g., flavor and aroma compounds) can be added back to the beverage. The required temperature can be reduced by the application of vacuum distillation (VD) at temperatures between 30°C and 60°C (Brányik et al., 2012). This helps to reduce the undesired alteration of flavor compounds and reduces the amount of energy required for the process. Further advantages of this process are the complete removal of ethanol from the beverage, the opportunity to sell the extracted ethanol and potential automation of the process (Branyik et al., 2012). For the production of alcohol-free beer on the industrial scale, VD is applied frequently but there can be serious alterations in aroma compounds compared to alcohol containing beer, summarized by Branyik et al. (2012).

For the production of alcohol-free wine, the spinning cone column (SCC) VD is one of the most commonly applied methods today (Belisario-Sánchez et al., 2012). A maximal recovery of flavor components is required, and this can be achieved by SCC. But the characteristic aroma of alcohol-free wine is not the same as its alcohol containing version. Belisario-Sánchez et al. (2012) proposed the utilization of alcohol-free wine as an antioxidant beverage or as a basis for mixed beverages.

Membrane-based methods comprise the dialysis/diafiltration and the RO. They offer the advantage of a comparably low energy demand. Furthermore, there are no undesired, thermally induced chemical reactions. Hence, flavor stability is high when ethanol is removed by membrane-based methods.

The driving force in dialysis/diafiltration is the concentration gradient between the alcohol containing beer and an alcohol-free dialysate (water). The applied membrane acts as a molecular sieve. Water and ethanol can pass through the membrane. To avoid the osmosis of water from the dialysate to the beer, filtration is conducted under pressure. Ethanol permeates to the dialysate. If the process is conducted in counter-current mode, effective removal of ethanol can be achieved. Unfortunately, membranes are also permeable to low-molecular weight aroma substances, such as ethyl-acetate, isoamyl-acetate, 2-methyl-propanol, or 1-propanol. These are also removed from the beer by this method, which can lead to severe aroma alterations. As an alternative, RO can be applied. Using pressures between 2 and 8 MPa and dense, asymmetric RO membranes leads to an efficient removal of alcohol. But also in this process, many aroma substances are removed from the beer, but in a smaller amount than in dialysis (Brányik et al., 2012). Due this fact, RO is one of the industrially applied methods for Ent alcoholization of wine.

Pervaporation: This separation process is a combination of permeation and evaporation. Due to its high selectivity, this technology is very promising for separation of difficult mixtures (Roy and Singha, 2017). The product does not need to be heated, and thus there is no danger of altered flavor profiles due to chemical reactions. Especially for the reduction of substances present in low concentrations, PV is a very good technology. Therefore, it can be applied for the recovery of aroma substances in the food industry. The process is strongly dependent on a suitable membrane, and there are many different reports that discuss different PV materials in detail. Permeation through the membrane can occur in a passive or in an active way. The passive permeation is driven by gradients in concentration between feed and permeate. The active transport is the result of a chemical reaction between the permeating substance and a component of the membrane (Roy and Singha, 2017).

Up to now, PV membranes are very costly. This fact limits their application in industrial scale, but there are significant enhancements in this technology including the increased stability of the membranes. Due to the high selectivity and the advantage of no thermal input, it can be expected that PV will be applied in many industrial processes in the coming years.

Catarino and Mendes (2011) suggest for dealcoholization of beer a two-step process. In the first step, PV is applied to extract the volatile compounds, which are then captured. In the second step, SCC is used to remove ethanol from the retentate. Afterwards, the captured aroma substances from the first step are introduced back into the retentate. Furthermore, some untreated beer is blended with the alcohol-free beer to achieve a beer with low alcohol content (<0.5% (v/v)) and optimal flavor profile. Takács et al. (2007) reported about the successful dealcoholization of wine by PV resulting in an alcohol-free wine with very pleasant sensorial properties. They calculated the costs for PV of 0.165 € per liter of wine, which may be acceptable for a high-price product like wine, but indicates also the high costs of this process caused by the high costs for the membranes.

3.2.2.1 Fermented Lemonades

Alcohol-free lemonades can be produced with microorganisms that do not produce alcohol as a fermentation product. One example is the "Bionade" (Bionade GmbH, Germany), a beverage produced with the bacterium *Gluconobacter oxydans*. *G. oxydans* belongs to the family of Gram negative acetic acid bacteria and is an obligate aerobe (Sievers and Swings, 2005). The natural environment of *G. oxydans* comprises flowers, fruits, and honey. Due to its incomplete oxidation of many substrates, it is of great interest for beverage production. For example, glucose is mainly oxidized to gluconic acid. This is a very rapid reaction because it is performed extracellularly, and the substrate does not need to be taken up by the cell. *G. oxydans* has several dehydrogenases in its periplasm. Therefore, no transport of the substrates and products into and out of the cell are required (Fig. 3.1, Deppenmeier et al., 2003).

Formation of gluconic acid offers the advantage for the consumer due to its potential cancer preventing effect after oral application (Kameue et al., 2004, 2006). In addition, exopolysaccharides are produced by *G. oxydans* that exhibit prebiotic properties (Mountzouris et al., 1999). During the development of fermented beverages, the formation of exopolysaccharides may result in challenges with the required downstream processing. Exopolysaccharides increase viscosity and they readily block filters. Hence, in early stages of beverage development, suitable strains should be selected, taking the filtration process into account.

No ethanol is produced during the fermentation process with *G. oxydans*. Besides acetic acid bacteria, lactic acid bacteria are suitable for the production of non-alcoholic fermented beverages. They are also able to synthesize exopolysaccharides with their discussed advantages and disadvantages. Furthermore, yeasts can also be used for the production of NAB. Promising yeast candidates include *Kluyveromyces* sp. Selected strains are able to produce compounds that exhibit a nice fruity flavor (Bader, 2008).

In case of the mentioned "Bionade," flavor substances are added to achieve the desired aroma after the fermentation process. Other examples for alcohol-free fermented beverages are Kombucha, Kvass (Basinskiene et al., 2016), Kefir, Sima, Bouza, Hardaliye, Shalgam drink, or Ayran (Altay et al., 2013). In the literature, some of the alcohol-free beverages we mention here are also categorized as

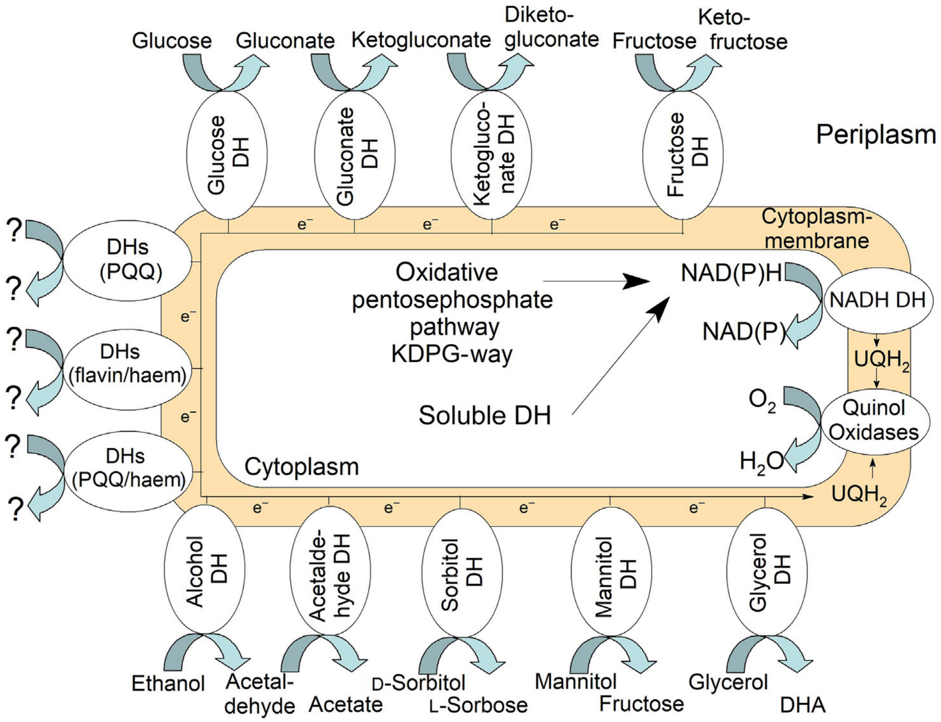

Fig. 3.1 Dehydrogenases of *Gluconobacter oxydans* oxidation of different substrates by dehydrogenases located in the periplasm of *G. oxydans*. Modified from Deppenmeier, U., Hoffmeister, M., Prust, C., 2003. Biochemistry and biotechnological applications of Gluconobacter strains. Appl. Microbiol. Biotechnol. 60, 233–242. doi:10.1007/s00253-002-1114-5.

alcoholic beverages. This designation can be explained by the use of altered fermentation conditions that lead to alcohol-free beverages for certain products when the fermentation time is short. Increased time of fermentation may lead to the production of an alcoholic beverage with the same substrate and the same participating microorganisms.

3.2.2.2 Fermented Milk Products

Yogurt and kefir are dairy-based fermented products typically fermented by mixed microbial culture. Milk is the basis for these products, and the fermentation processes for each share some similarities but also have their differences. Both yogurt and kefir have been linked, albeit anecdotally, with antimicrobial properties and other health benefits to the consumer.

Yogurt is typically fermented with a defined mixture of starter culture bacteria. Common starter culture members include *Streptococcus salivarius* (subsp. *thermophilus*) and *Lactobacillus delbrueckii,* and the fermentation and interactions between these microbes are well-documented. *S. salivarius* and *L. delbrueckii* stimulate each other's growth and these species readily exchange nutrients: *S. thermophilus* provides formic acid, folic acid, and fatty acids, while proteolytic activity by *L. delbrueckii* provides amino acids (Smid and Lacroix, 2013; Sieuwerts et al., 2010;). The activities of the microbial consortium in yogurt result in the biochemical conversion of milk products to make key yogurt compounds, specifically: (1) conversion of lactose into lactic acid, (2) hydrolysis (proteolysis) of casein into peptides and amino acids, and (3) breakdown of lipids into free-fatty acids. In conventional yogurt, proteolytic activity is undertaken by *L. delbrueckii*, allowing for *S. salivarius* to use the proteolytic products as carbon sources and thus obtains the amino acid requirements of the feeder species. The coculltivation of these two organisms also has an effect on the concentration of aroma compounds. Monocultures of either *S. salivarius* or *L. delbrueckii* exhibited significantly lower concentrations of the aroma compound dimethyl trisulfide (DMTS) as compared to cocultures of the organisms (Smid and Lacroix, 2013).

Kefir is a fermented beverage that originates from the north Caucasus Mountains. Like with yogurt, it is prepared by the inoculation of milk (cow, sheep, goat milk, or other) with microbial starter cultures. Unlike with yogurt, the starter cultures are present in kefir grains (Satir and Guzel-Seydim, 2016; Altay et al., 2013). These kefir grains contain a coculture of bacteria and yeasts, as well as a water-soluble polysaccharide called kefiran. Kefiran, a branched polymer consisting mostly of glucose and galactose (Kooiman, 1968), adds a creamy texture and mouth feel to the final beverage product. The kefir grains contain a stable and specific microbiota encapsulated in the polysaccharide matrixes (Leite et al., 2013). Kefir grains are typically inoculated into pasteurized milk, incubated for approximately 24 h, and then removed. These grains can then be reused to inoculate a fresh milk culture, as they still contain large numbers of primary culture organisms.

The kefir microbiota contains lactic acid bacteria and yeasts, as well as other microorganisms. These organisms work as a cooperative coculture to promote the growth and survival of the consortium and all its members. The yeasts in the culture typically provide growth factors, such as vitamins and amino acids, and the bacterial end products (i.e., organic acids, etc.) are used by yeasts for carbon and energy sources (Viljoen, 2001). Kefir is characterized by a flavor that is attributed to yeast activity. This includes the characteristic effervescence of the beverage, which is the result of CO_2 production by yeast. The predominant lactose-fermenting yeast species present in most kefir grain samples are *Kluyveromyces lactis*, *Candida kefir* (*Kluyveromyces marxianus*), and *Debaryomyces hansenii.* Non-lactose fermenting yeast can also be found, including *S. cerevisiae*, *Pichia fermentans*, and others (Leite et al., 2013). The prokaryotic component of the kefir grain consortium includes *Lactobacillus* species, *Lactococcus* species, *Streptococcus thermophilus*, *Leuconostoc mesenteroides*, and others (Leite et al., 2013, 2012).The complex interactions among all the microbial species present in kefir grains are not well understood. The complex interactions among the different microbial species in kefir grains are important for both the quality of the final product as well as for the survival of each member of the coculture. It has been shown that, when the kefir bacteria are separated and removed from the grain, the yeast still present in the grain grew at a poorer efficiency (Rattray and O'Connell, 2011). This underscores the importance of the synergy among the microbial consortium members present in kefir grains.

To produce yogurt, starter culture, that is, a mixture of *S. salivarius* and *L. delbrueckii*, is added to pasteurized milk. The starter coculture ferments the lactose in the pasteurized milk, producing lactic acid, which thus gives yogurt its characteristic tangy flavor. Another flavor compound that is produced during yogurt fermentation is acetaldehyde, thought to be synthesized mainly by *L. delbrueckii* (Beshkova et al., 1998). Because acids are being produced by the starter cultures and accumulate in the product, the milk proteins coagulate and set, which ultimately gives yogurt its viscosity and creamy consistency.

The presence of lactic acid bacteria in yogurt suggests that consumption of the product has its health benefits. Because traditional yogurt contains live cultures of bacteria, it has traditionally been used as a delivery mechanism for probiotic organisms. However, the yogurt starter bacteria, *S. salivarius* and *L. delbrueckii*, do not survive the passage through the gastrointestinal tract. Because of this phenomenon, manufacturers will often add bacteria like *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Bifidobacterium* sp. to yogurt to bolster its concentration and diversity of probiotic organisms (Ashraf and Shah, 2011).

Several studies have attempted to demonstrate the health benefits of yogurt consumption in a variety of ways. Yogurt consumption has been suggested as a treatment for antibiotic-associated diarrhea (Beniwal et al., 2003), as well as for increasing the chances of eradication of *Helicobacter pylori* infection (Adolfsson et al., 2004). It has even been suggested that yogurt has antitumor and anticancer activity (Perdigon et al., 1994; Rachid et al., 2002). In “normal” (i.e., perceptively healthy) hosts, yogurt was shown to stimulate immune cells that are associated with the gut (Perdigon et al., 1994). Kefir also has been associated with health benefits, including those resulting from the presence of similar probiotic bacteria and other organisms in the kefir grains. Like with yogurt, kefir has been suggested to inhibit the growth of pathogenic organisms that can cause gastrointestinal distress

(Carasi et al., 2014; Zhu et al., 2014). Studies also claim to demonstrate that kefir consumption can be protective against cancer (Chen et al., 2007; Sarkar, 2007).

3.2.2.3 Kombucha

Traditionally, Kombucha is a nonalcoholic beverage (<0.5%(v/v)), derived from an aerobic cofermentation process by acetic acid bacteria and yeast, based on different teas and sugar. During the fermentation process, acetic acid and gluconic acid are produced. In the traditional process, during fermentation, a layer of bacterial cellulose is formed. This biofilm is swimming on the surface and limits the oxygen transfer. For industrial production of Kombucha, this formation of the biofilm is a disadvantage because it is difficult to remove from the fermentation vessel after the fermentation process. To overcome this issue, selected strains are fermented in a special sequence. Chakravorty et al. (2016) applied next generation sequencing to identify all participating microorganisms during traditional Kombucha fermentation. They found *Candida* sp. to be the dominant yeast while *Acetobacter* sp. was the most prevalent bacterial genus. Furthermore, they found different compositions of microorganisms in the liquid and the biofilm phases. While 88.5% of all bacteria the biofilm belonged to *Acetobacter* sp. and only few other bacteria could be identified, many different yeast strains could be identified in the biofilm. In the liquid phase a much higher diversity was observed, especially among the bacteria.

Even if Kombucha is already being produced in industrial processes, further laboratory-scale research is still going on. Coton et al. (2017) identified several variations in the microbial composition, but found that there are also core microorganisms that are prevalent in many of the examined fermentation processes. Furthermore, the authors observed differences in microbial strains depending on the type of tea (green or black) used in the ferment. Traditionally, it is said that the consumption of Kombucha exhibits health benefits, but this claim is still uncertain and convincing scientific evidence confirming this is missing (Coton et al., 2017).

3.2.3 Detection of Flavor Compounds: Beverages in the Age of Advanced Biochemistry

Due to the high relevance of flavor substances in fermented beverages for the consumer, sophisticated methods for their analysis have been developed.

Flavor compounds not only play a role in the enjoyment of fermented beverages like wine, but also play a significant role in evaluation of quality and detection of spoilage. Compounds that affect flavor occur in a wide range of concentrations in wine and other beverages, from nanogram per liter to gram per liter quantities. In wine, considerable amounts of these flavor compounds are already present in the original grapes used, but many compounds are introduced or altered as a result of the fermentation process. Classes of compound that impart flavor to wine include: (1) organic acids, (2) volatile organics (e.g., alkanes, aldehydes, higher alcohols, and others), and (3) various other compounds like furanoids and/or pyrenoids (Schreier, 1979). Given the advances in technology in recent years, beverage manufacturers and scientists can detect the compounds with accuracy, which impart flavor profile on specific beverages.

Many flavor and aroma compounds in wine are volatile chemicals, and thus are easily detectable by gas chromatography (GC). Indeed, hundreds of compounds have been detected in wines using this method. Identification of unique flavor and aroma compounds can be performed by mass spectrometry (MS), with tandem GC/MS being a valuable technique for differentiating between flavors among different wines. In some cases, high-performance liquid chromatography (HPLC) has been used as a detection method. How can individual wine chemicals be "matched" with specific odors they may impart to the beverage? For this, the researcher can use the combination of GC and olfactometry (GC/O, also known as CHARM analysis (Jackson, 2009). GC/O usually relies on human evaluators to detect volatile compounds that elute from a GC. Assessors sniff the eluate in order to determine the presence and quality of an odor using a specifically designed odor port (Delahunty et al., 2006). Guth et al. (1997) used GC/O to detect and characterize flavor and aroma compounds in Gewürztraminer and Scheurebe wines. Ethyl 2-methylbutyrate, 3-methylbutanol, and 2-phenylethanol were some of the compounds shown to possess high flavor dilution factors in both types of wine. Guth also characterized a previously unknown compound (initially called "wine lactone") with high flavor dilution factor in both wines, 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one. The compound exhibits a sweet, "woody" and coconut-like odor. Similarly, flavor and odor profiles of Australian shiraz wines from different regions were compared using GC/O. Wines from the warmer of the two regions possessed higher concentrations of ethyl propionate and dimethyl sulfide, and wines from the cooler region showed higher concentration of rotundone (a "peppery" flavor compound) and 2- and 3-methylbutanoic acids (Mayr et al., 2014).

Ragazzo-Sanchez and coworkers coupled GC with an electronic nose to detect off-flavors in red wine. The wine was de-alcoholized and dehydrated using a backflush technique. Five off-flavor compounds were successfully detected by this method: *n*-hexanol, oct-1-en-3-ol, 2,4,6-trichloroanisole (TCA), 4-ethyl phenol, and ethyl acetate (Ragazzo-Sanchez et al., 2005). Dziadas and Jelen examined the release of bound flavor compounds in grape juice using acid and enzymatic hydrolysis, with subsequent detection by GC/MS. Levels of terpenes identified as flavor agents were shown to increase post-hydrolysis, with acid hydrolysis yielding more terpenes than enzyme-mediated hydrolysis. This study serves as a reminder of the advantage of the low pH of wine in enhancing its overall flavor characteristics (Dziadas and Jeleń, 2016). Flavor and aroma compounds in toasted oak have also been studied, to demonstrate what types of compounds are imparted to wine during storage in oak barrels. GC/MS was used to detect eugenol, furfural, vanillin, and other compounds in toasted oak battens (Li et al., 2015). Mayr et al. (2015) used GC/MS-MS to detect oxidative off flavor compounds in wine. Using this method, methional, 2-phenylacetaldehyde, and 3-methylbutanal were detected in wines. These oxidized off flavor compounds were most prevalent in wines that had been sealed with synthetic closures, compared to those that were sealed by screw-cap or natural cork. Recently, GC/O was used to detect off-flavor compounds present in faulty cork stoppers. Raw cork material was soaked in water, and the compounds that leached out were detected and analyzed. The group detected different compounds that were associated with distinct odors: geosmin and 2-methylisoborneol were detected as “moldy” or “cellarlike” odors, 3-isopropyl-2-methoxypyrazine and 3-isobutyl-2-methoxypyrazine were detected and associated with “green” (i.e., vegetal) attributes. Two compounds were detected by this method that the authors declared could be used as markers for bad cork taint. Across all cork subgroups tested, TCA and 3,5-dimethyl-2-methoxypyrazine (MDMP) were detected (Slabizki et al., 2015). Sadoughi et al. (2015) examined off-flavors in Shiraz, Cabernet Sauvignon, and Semillon wines caused by the presence of contaminating fungi. Compounds such as geosmin, 2-methylisoborneol, 3-octanone, fenchol, and others were detected. Many compounds were detected in both grape homogenate (from grape bunches infected by different molds) and wine. Off-flavor compounds were detected by GC/MS.

Not only can flavors and odors in wine be detected with modern biochemical techniques, but pigments can also be examined by these different methods. Many types of pigment compounds in red wine are polyphenolic compounds, which have been shown to have value as antioxidants and have even been demonstrated to have potential health benefits. Oliveira et al. (2015) detected many different polyphenolic anthocyanins and pigment compounds in red grape pomace using LC/MS and MALDI-TOF.

Volatile compounds, whether flavor or off-flavor compounds, have been extracted from the liquid wine or from the headspace gas and detected by a variety of methods. Another method of extraction was devised in the 1990s called solid-phase microextraction (SPME) and is frequently used in current studies examining flavor and aroma compounds. The advantage of the technique is that concentration and extraction are combined in a single step. Like traditional extraction methods, SPME can occur either from the liquid or from headspace gas (Robinson et al., 2014). SPME uses fibers that are coated with either polymers or sorbents. These fibers can extract volatile compounds from liquid or gas phases (Mitra, 2003). Different types of coatings (e.g., polar or nonpolar) can be used, depending on the chemical nature of the compounds of interest to be extracted. Once the fiber has extracted compounds, it can be inserted into a chromatograph for desorption, detection, and quantitation (Vas and Vékey, 2004). Slaghenaufi et al. (2014) used SPME to extract megastigmatrienone isomers from aged wines. Megastigmatrienone is a flavor compound in tobacco, but is often associated with wine. The isomers were analyzed in this work by GS/MS. It was shown that levels of megastigmatrienone increased with aging of the wine. Volatile compounds in must and wines made from interspecific hybrid *Vitis* (IHV) grape varieties were extracted by SPME and analyzed. IHV grapes are of interest in cold regions, such as the northern United States and Canada, as they are cold- and fungus-resistant varieties. Several compounds, including hexanol, isoamyl acetate, *p*-vinylguaiacol, and others were detected in juice and wines from IHV grapes in Quebec (Slegers et al., 2017). SPME was also used to extract off-flavor compounds from grape homogenates and wines affected by different fungal diseases (Sadoughi et al., 2015).

Other detection methods have been used to detect important chemical compounds in wine. Marri et al. developed an ELISA method to detect diacetyl, a flavor compound sometimes found in beer and often associated with off-flavors in wine. The resulting assay can detect diacetyl in micromolar concentrations (Marri et al., 2017). However, the industry in general primarily uses the methods described above for valuable compound detection, due to the sensitivity, repeatability, and reliability of the assays.

3.3 Development of a Beverage by Coculture Fermentation

In the first steps of development, the general framework of the beverage has to be defined. Which substrate will be used? What kind of ingredients should be in the fermented beverage? In the described process, beer wort should be used as substrate to open up new markets for breweries. The produced beverage should contain less than 0.5%(v/v) of ethanol because it would belong to the “alcohol free” beverage category, and the organic acids lactic acid and gluconic acid should be present in the beverage due to their health promoting effects. Of course, the beverage must have a pleasant flavor and no or undetectable off-flavors.

To achieve the goals of this beverage development (low/no alcohol, presence of certain organic acids), suitable microbial strains should be selected to allow for achievement of these goals. The strain of the lactic acid bacteria should belong to the family of homofermenting bacteria, because acetic acid should not be prevalent in the beverage due to its unpleasant aroma. A strain of *Gluconobacter* sp. was selected regarding its production rate of gluconic acid using beer wort as a substrate. During the production strain selection process, not only the production rates and final concentrations of organic acids were evaluated, but also the sensory properties (Bader, 2008). Any kind of off-flavor compounds were avoided. For the selection of a suitable yeast strain, it was necessary to choose a strain with a low fermentation rate in beer wort and the ability to produce compounds that result in a pleasant flavor (Bader, 2008).

3.3.1 Optimization of Culture Conditions in Pure Cultures

After the described screening for suitable production microbes, three strains were selected: *Lactobacillus* sp., *Gluconobacter* sp., and *Kluyveromyces* sp. For these strains, the influence of different fermentation conditions (pH, temperature, oxygen availability, substrate concentration) was investigated. Optimal fermentation temperature for *Lactobacillus* sp. was 37°C, for *Gluconobacter* sp. 26°C. While the lactic acid bacterium requires anaerobic conditions, *Gluconobacter* sp. needs oxygen for the production of gluconic acid. During this optimization of pure culture fermentations, it was investigated that the selected *Gluconobacte*r strain is able to hydrolyze maltose extracellularly under anaerobic conditions (Bader, 2008). This results in an increase of glucose concentration in the medium.

The chosen yeast strain was very sensitive to altered fermentation conditions. It was found that a high glucose concentration improves flavor formation. This can be explained by the regulation of the formation of sensory active esters from glucose (Verstrepen et al., 2003). Furthermore, the yeast started to produce unpleasant, bitter off-flavors when glucose was depleted. This fact is important for the developed fermentation product, as it means that ample glucose must be available throughout the whole fermentation process. The concentration of remaining sugars is also dependent on the amounts of organic acids produced. Sugars and acids influence each other in the development of sensory properties. There has to be a specific sugar-to-acid ratio to produce a pleasant flavor. If the amount of produced acids increases, more sugar is required to achieve a pleasant flavor. Flavor formation by the selected *Kluyveromyces* strain was also dependent on the pH-value (Table 3.3). This indicates that there are several interacting parameters for the coculture fermentation process that have to be taken into account for the development of the fermentation process.

The pH-dependence of the sensory properties is very critical due to the desired production of organic acids by the selected bacteria, followed by decreasing pH values.

The investigations regarding the influence of process parameters described here are important for the development of a controlled coculture fermentation strategy.

3.3.2 Development of the Defined Coculture Fermentation Process

The coculture fermentation process should result in a pleasant beverage and it should offer brewers the possibility to broaden their choice of products. It is for these reasons that beer wort had been chosen as a substrate. Organic acids and the desired flavor should be produced by the controlled cocultivation of *Gluconobacter* sp., *Lactobacillus* sp., and *Kluyveromyces* sp. Combining the results from the described single culture optimizations resulted in a three-phase cocultivation process (Fig. 3.2). In the first aerobic phase, gluconic acid was produced. In the second phase (anaerobic), new beer wort was added. The maltose present in this substrate was hydrolyzed, and the glucose concentration increased. A high concentration of glucose is required for flavor formation by the yeast in the third phase of fermentation. During this anaerobic phase lactic acid is also produced.

Table 3.3 Sensory Evaluation of a Fermentation Product Produced by Fermenting Beer Wort With a Selected Strain of *Kluyveromyces* sp.

Start pH	End pH	Smell	Aroma
5.7	4.9	Sweet, fruity	Sweet, no off-flavor
4.8	4.6	Sweet	Fresh, sweet, no off-flavor
3.9	3.9	Sweet	Fresh, no off-flavor
3.2	3.2	Ethanol, yeast	Sour, unpleasant, cheese

Inoculation rate was 10^7 cells/mL, fermentation temperature is 26°C, and fermentation time is 96 h (Bader, 2008).

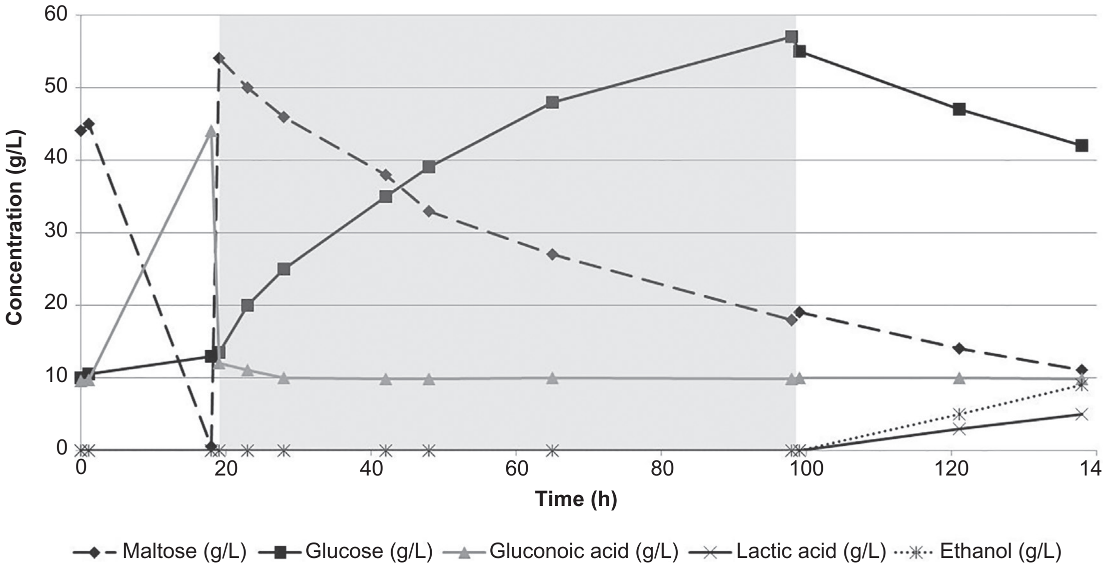

Fig. 3.2 Coculture fermentation process for the production of a beverage on the basis of beer wort. Fermentation temperature was 26°C, 0 h: inoculated with *G. oxydans*, 0–19 h: aerobic conditions, >19 h: anaerobic conditions, 98 h: inoculation with *Lactobacillus* sp. and *Kluyveromyces* sp. (according to Bader, 2008).

Temperature in all phases of fermentation was 26°C, which was the optimal temperature for *Gluconobacter* sp. and *Kluyveromyces* sp., while the metabolism of *Lactobacillus* sp. was reduced to prevent the formation of too much lactic acid. The development of this fermentation strategy was conducted in a 5-L bioreactor and transferred to a 150-L bioreactor with a working volume of 50 L.

Afterwards, the whole fermentation process was transferred to a brewery. Compared to a traditional beer production process, it was necessary to proliferate additional biomass in additional precultures. After adaption of the required fermentation equipment, a comparable coculture fermentation process was conducted in 400-L scale under brewery conditions resulting in a fruity beverage with pleasant sensory properties. The advantage of the developed cocultivation process over single yeast fermentation can be seen in the results of aroma analysis (Table 3.4). The presented results were achieved by GC-MS analysis of two different fermentation products. In the first sample, a pure culture of the selected *Kluyveromyces* strain had been used in fermentation. In the second sample, a coculture of *Gluconobacter* sp., *Lactobacillus* sp., and *Kluyveromyces* sp. had been applied. In both samples, 12% beer wort was used, and fermentation time was 48 h at a temperature of 26°C.

The results illustrate the advantage of coculture fermentation processes. Many flavor-active substances are produced in higher amounts

Table 3.4 Comparison of Flavor-Active Substances Produced During Pure Culture (*Kluyveromyes* sp.) or Coculture (*Gluconobacter* sp., *Lactobacillus* sp., and *Kluyveromyces* sp. in Beer Wort at 26°C and Fermentation Time of 48 h (According to Bader, 2008)

	Concentration in ppm	
Substance	***Kluyveromyces* sp.**	**Coculture**
2-OH-3-Me-butyric acid	n.d.	1.3
4-Methyl-pentanic acid	0.3	2.7
3-Methyl-pentanic acid	0.6	1.7
Furaneol	0.6	0.7
Phenylethanol	n.d.	0.6
Furaneol isomers	n.d.	1.5
2-Phenyl-acetic acid	0.9	1.1
Phenylethyl acetic acid	1.0	6.3
2-Phenyl-propionic acid	1.1	3.7

in coculture compared with single cultivation of the yeast. An explanation for this could be the prevalence of more precursor substances for flavor production. Furthermore, altered substrate concentrations may result in different gene activations, resulting in the formation of other flavor compounds. Another possibility is the communication between the microorganisms in the coculture by chemical molecules (i.e., quorum sensing (QS)).

Coculture fermentation processes can be applied in berverage production, but this process also offers the possibility for the biotechnological production of natural flavor substances by fermentation.

The coculture fermentation had to be stopped immediately after 140 h (Fig. 3.2). Therefore, a pasteurization step must be conducted. It was not possible to cool down the fermentation product because, in this case, microorganisms started to produce extracellular polysaccharides (Bader, 2008). This negatively influenced the mouth feel of the product. Furthermore, filtration was not possible due to the increased viscosity. After pasteurization, a filtration step is necessary to clarify the product and to reach storage stability.

3.3.3 Modern Detection Methods for Research in Coculture Fermentations

For the production of beverages with a reproducible flavor profile, a controlled coculture fermentation strategy is required. Therefore, it is of great interest which microorganisms are prevalent in traditional mixed culture fermentations. Furthermore, their metabolic activity in the fermentation process should be known and understood in order to design a controlled process. In addition, the interactions of microorganisms and human intestinal cells are of high relevance for the development of accepted probiotics with proven health-benefiting effects. One of the suitable methods for the identification of microorganisms in mixed cultures is high-throughput sequencing. A collection of results of microbial mixed cultures in beverages and many other samples can be found at the website: https://www.ebi.ac.uk/metagenomics (accessed: October 25, 2017). Another online database of microorganisms used in food industry has been published by Parente et al. (2016).

Mayo et al. (2014) discussed the application of modern "-omics" technologies, such as metabolomics, proteomics, and glycomics, to achieve the required insight in the role of microorganisms and flavor formation. Denaturing high-pressure liquid chromatography (DHPLC) and next-generation sequencing had been used to investigate the microbiome during vinegar production by Trček et al. (2016).

The application of these modern techniques for the determination of useful microorganisms in the food industry indicates the high relevance of fermented food. There is an increasing awareness of consumers that traditional fermented food and traditionally consumed fermented beverages can contribute to human health. For the industrial production of these products controlled cocultivation methods should be developed.

3.4 Filtration of Fermented Beverages

Wine and beer are two of the most consumed beverages in the world today. Both are very ancient beverages, with their origins dating back thousands of years (Esmaeili et al., 2015). In modern times and thanks to the development of many advanced technologies, brewing and winemaking have become exacting industrial processes, which have allowed producers to minimize wasted and contaminated batches, thus stabilizing the quality of the product. Post-fermentation, both beer and wine appear cloudy due to large quantities of suspended solids present (Ambrosi et al., 2014). Removal of these solids is performed by a process called clarification (Robinson and Harding, 2006). For wine, the solids removed are unstable proteins and other macromolecules, as well as potential aggregates that would significantly decrease the quality of wine. This is important as wine should retain stability and clarity during storage (Mierczynska-Vasilev and Smith, 2015). Clarification is an important step in beer making, even with the recent popularity of small batch, unfiltered beers (e.g., saison or wheat beers). Many types of beer that are widely consumed, including lagers and pilsners, are expected to be clear (Ward, 2000). Thus, in the latter cases, clarification of beer is required to give it the necessary visual appeal.

Beer is the most consumed alcoholic beverage in the world with 1.93×10^{11} L consumed in 2016 (https://www.statista.com/statistics/270275/worldwide-beer-production/). With the exception of a few unfiltered, traditional beers, turbidity in the product is considered a significant shortcoming in quality (Braun et al., 2011). Many applications of filtration focus on clarifying the final beer. However, filtration can be used in a variety of circumstances during the beer production process. Filtration can be combined with other separation processes. In the first step, most of the biomass is removed and then a filtration process is applied to remove residual biomass and to achieve a microbially stable product with a long shelf life.

Traditionally for clarification of beer, diatomaceous earth (a.k.a, kieselguhr) is used. Kieselguhr is an important and commonly-used filter aid for many applications due to its high inner porosity (Braun et al., 2011). It can be applied to the pre-coating of the filter to achieve an efficient and rapid filtration process, or it can be added continuously during the filtration process to prevent an undesired compression of the compressible yeast.

There are indeed concerns over the use of this particular filter aid, including the amount of beer-soluble metals present in kieselguhr, which will diminish beer quality. Also, the WHO has ascertained that the silica present in kieselguhr can cause lung disease, thus spent diatomaceous earth sludge is highly regulated in terms of disposal (Braun et al., 2011; Cimini and Moresi, 2014). The disposal of kieseguhr became more and more expensive due to the health issues. As a result, other methods of filtration have been sought in recent years.

As an alternative to kieselguhr, other filter aids have been examined for beer clarification. Different forms of cellulose (fibrillated fibers, microfibers and fibers with lengths in the tenths of mm scale) have been tested for their filtration qualities in this application. Cellulose is attractive for this purpose because it can be regenerated multiple times, its disposal is relatively easy, bears no health risks, and the raw material is renewable. Unfortunately, cellulose alone delivers unsatisfactory performance in beer clarification. However, with the addition of a filter aid like silica sol, the filtrate haze was reduced significantly in both wort and green beer filtrations (Braun et al., 2011). Other polysaccharides have been used in beer clarification. Chitin and chitosan, both natural polysaccharides from crustacean shells, were examined as flocculants in both lab scale (4L) and industrial scale (1000L+) settings. In previous studies, chitin, chitosan and derivatives were successfully used to clarify wine and fruit juices (for a review on this matter, see Rocha et al., 2017). Both chitin and chitosan were shown to be effective in flocculation, equal in performance to flocculants typically used in the brewing industry. Both polysaccharides also showed superior solids removal compared to other flocculants. Overall, the authors of the study concluded that chitin and chitosan are superior flocculants compared to commonly used additives currently available (Gassara et al., 2015). Another type of polysaccharide, carageenan, was studied for its ability to clarify beer wort and reduce the amouth of kieselguhr used in the clarification of the final beer product. Optimal results could be obtained when clarifying beer from carageenan pretreated wort by filtering using 8X less kieselguhr (Poreda et al., 2015). Also, Linforth et al. demonstated that Galena hop extracts, more specifically hop proanthocyanidins could act as fining agents in beer. These compounds were shown to significantly reduce the optical density of beer after 2h of treatment. The ability to promote flocculation has been shown in extracts of other types of hops, as well (Linforth et al., 2015).

In recent years, an increasing number of membrane-based filtration processes have been used for beer clarification. Most of these processes used membranes consisting of of polyethersulfone (PES), but there are also some filtration modules made by ceramic membranes on the marked. These membranes can be cleand by NaOH or vapor, easily due to their high chemical and thermal resistance but the required investments are much higher than filtration units with PES membranes. This kind of clarification method for beverages is ideal because it maintains the quality of beverages, guarantees sterility (if the proper membrane is used) and bypasses the need for potentially toxic kieselguhr sludge buildup (Esmaeili et al., 2015). Membrane separation offers advantages over traditional technologies, such as lower thermal impact on products, capable separations, moderate energy consumption, and modular design (Ambrosi et al., 2014). Clarification of beer was assessed on the pilot scale using pretreatment by adsorption of particles and polyphenolic compounds to polyvinylpolypyrrolidone granules followed by crossflow microfiltration through ceramic hollow fiber membrane cartridges. The resulting permeate beer was haze-free at cold temperatures without the use of traditional filter aids (Cimini et al., 2014). Cross-flow filtration of lage beers using ceramic membrane cartridges was shown to be more effective in production performance than the currently available filtration setups (Cimini and Moresi, 2016a). As a follow-up work, the same group utilized a laboratory-scale clarification process using only the cross-flow microfiltration method. The key to the group's design of the filtration process was, like with many filtration processes, based on control of fouling and cake resistance. Once this aspect of the process was controlled, the permeation flux for this process was shown to be higher than any other currently available filtration process (Cimini and Moresi, 2014). Membrane pore size has been shown to affect the overall beer quality. Membranes with pore diameters ranging from 0.2 to 1.4 μm were examined. The filtration combination of a 1.4-μm pore size, followed by a finishing step using 0.45–0.6-μm pores yielded the best results, producing a haze-free beer with optimal color, alcohol content, and stability (Cimini and Moresi, 2016b).

During the filtration of fermentation products produced via coculture fermentation, there can be severe issues if microbially produced polysaccharides are present. These polysaccharides will rapidly foul the filter, preventing an economic filtration process. Enzymatic pretreatment or precipitation methods prior to filtration may help to overcome this problem.

The filtered product must be evaluated regarding flavor stability, possible haze formation, and microbial stability. Then, according to the evaluation results, the filling processes and suitable packaging materials has to be selected for the final process. The described development for the cocultivation process is presented in Fig. 3.3.

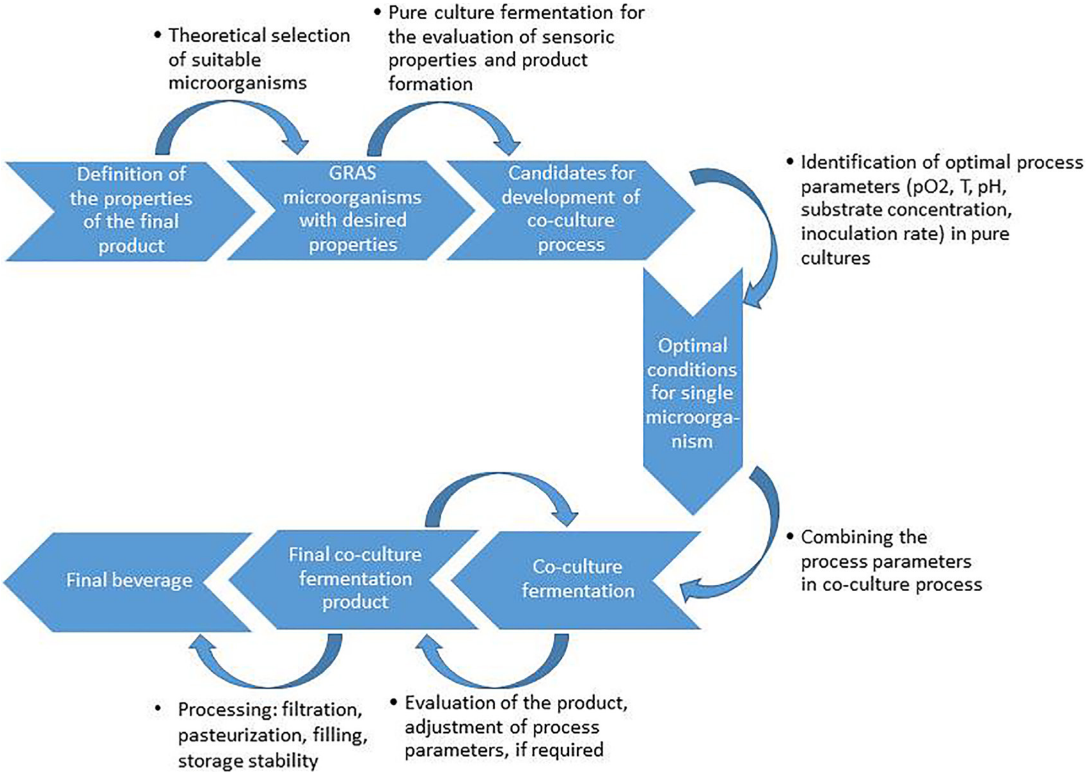
• Theoretical selection of suitable microorganisms
• Pure culture fermentation for the evaluation of sensoric properties and product formation
Definition of the properties of the final product
GRAS microorganisms with desired properties
Candidates for development of co-culture process
• Identification of optimal process parameters (pO2, T, pH, substrate concentration, inoculation rate) in pure cultures
Optimal conditions for single microorganism
• Combining the process parameters in co-culture process
Co-culture fermentation
Final co-culture fermentation product
Final beverage
• Processing: filtration, pasteurization, filling, storage stability
• Evaluation of the product, adjustment of process parameters, if required

Fig. 3.3 Scheme of the development of a fermented beverage gained by a cocultivation process.

3.5 Microbial Interaction in Fermentation Processes

In many fermented beverages, a decrease in pH value can be observed during the fermentation. It can be observed during co-culture fermentations, including those of kombucha or kefir, but also during the pure culture process of beer fermentation. The decrease in pH can be caused by produced organic acids, including lactic acid or acetic acid, or by the secretion of protons (e.g., by yeast). However, the decreasing pH value leads to a microbial stabilization of the product and prevents proliferation of undesired or even pathogenic microorganisms. Short-chained organic acids have inhibitory effects to microorganisms because they can change the internal pH value followed by inhibition of microbial enzymes (Salmond et al., 1984). Furthermore, it was reported that lactic acid and malic acid can disrupt QS strategies. It was observed that QS between pathogenic *E. coli* O157:H7 strain and *Salmonella typhimurium* could be interrupted by malic acid and lactic acid followed by the reduced virulence Almasoud et al. (2016). This can be explained because many pathogenic microorganisms coordinate the expression of virulence factors by QS.

As discussed above, many fermented beverages are produced by mixed culture or coculture fermentation processes. It can be assumed that there are several interactions between the participating microorganisms, often by so-called QS. QS is a chemical communication of microorganisms between members of their own species, as well as with other microbial species. Furthermore, chemical communication can occur with plants and even higher animals. QS between microorganisms can take place using several different chemical substances, such as *N*-acyl homoserine lactone (AHL), furanosyl borate diester, secreted small-peptide pheromones, γ-butyrolactones, farnesol, tyrosol, phenylethanol, and tryptophol (Albuquerque and Casadevall, 2012; Fleuchot et al., 2011; Waters and Bassler, 2005).

Also, eukaryotic cells are able to use QS mechanisms. Surette (2016) and Ismail et al. (2016) reported about epithelial cells that secrete QS moelcules comparable to bacterial QS molecules in order to communicate with the ruminal microorganisms. These examples indicate the complexity of the mixed culture in the intestinal system. Therefore, QS is a phenomenon that has to be taken into account for the evaluation of probiotic food products and beverages.

Many microorganisms are producing volatile, aroma-active substances, even if these substances have no direct benefit for the microorganism—at least according to first perception. Phenylethyl alcohol is one of these substances. It is produced by yeast and exhibits a strong flavor. The relevance of the substance becomes obvious when it is taken into account that phenylethyl alcohol is also a QS molecule. Park et al. (2016) could show that some QS molecules are produced by microorganisms in the presence of others. Similar results are also presented in Table 3.4. Hence, it can be expected that many more aroma-active substances are formed in mixed culture or coculture fermentation processes as compared with pure culture fermentations. For industrial beverage production, it is of great interest to determine all participating microorganisms and their interactions to achieve a controllable fermentation process and a reproducible product. Furthermore, it is of high relevance to investigate the interactions of microorganisms and the human consumer for the development of beverages with functional properties and beneficial influences on the human intestinal system and the human health.

Several traditionally fermented food products contain QS molecules and/or active microorganisms that are able to produce these substances in the gastrointestinal system. It was shown that, due to their increased concentration, the proliferation of Firmicutes was increased, and negative effects of antibiotic treatment could be minimized (Thompson et al., 2015).

3.6 Outlook of Fermented Beverages With Functional Properties

Human health is strongly dependent on the microorganisms in the intestinal system, and in recent years there has been a growing knowledge about the communications and interactions between microorganisms and human host. It seems that the traditional opinion of “healthy fermented food” is confirmed by modern science. This offers the possibility to develop fermented beverages with functional properties. Substrates can be enriched with vitamins and amino acids, and undesired phenolic substances can be reduced. At least as important as the substances present in the microorganisms themselves. The intake of living microorganisms can influence human health. Probiotic food products are thought to be advantageous for health and well-being, and there are many reports about their positive influence. In the last decade, many food companies tried to achieve a health claim by European Food Safety Authority (EFSA) but failed due to insufficient characterization of the microorganisms or insufficiently large group sizes in human studies. In March 2017, the first microorganism gained health claims by EFSA. For *Propionibacterium freudenreichii* W200 the following health claims are allowed due to its high production of vitamin B12 (Winclove, 2017):

1. Vitamin B12 contributes to normal energy-yielding metabolism.
2. Vitamin B12 contributes to normal functioning of the nervous system.
3. Vitamin B12 contributes to normal homocysteine metabolism.
4. Vitamin B12 contributes to normal psychological function.
5. Vitamin B12 contributes to normal red blood cell formation.
6. Vitamin B12 contributes to the normal function of the immune system.
7. Vitamin B12 contributes to the reduction of tiredness and fatigue.
8. Vitamin B12 has a role in the process of cell division.

It can be assumed that more claims will be authorized in the coming years. This offers the opportunity to develop new food products and beverages. Due to the increasing number of individuals who claim to be vegetarians or vegans, the consumption of fermented beverages can help to overcome the insufficient supply with vitamins in this group of people.

3.7 Conclusion

Fermented beverages have been produced since the beginning of recorded history, even long before mankind had any knowledge about participating microorganisms. Worldwide, many beverages are still produced by traditional fermentation recipes, and the gained fermented beverages contribute to the lifestyle of the consumer. Furthermore, the fermented substrates are protected from spoilage and can prevent the uptake of pathogenic microorganisms. Hence, fermented beverages help to keep humans healthy. There are not only alcoholic beverages but also a number of fermented beverages that contain only low amounts of, or even no ethanol, which is beneficial due to the health concerns about ethanol consumption.

During the fermentation process, a number of desired products like vitamins can be produced. This can help to overcome insufficient vitamin uptake, especially in the vegan diet. Hence, the old and traditional fermentation processes can be increasingly beneficial for the modern way of life with vegan diet.

Several traditional fermented beverages are being produced in industrial processes including beer, wine, kefir, ayran, kombucha, and many spirits, and there is still a lot of development in applied technologies and microbiology to produce fermented beverages in an economic way.

There is still much actual research being published about participating microorganisms and further insights in the interaction of produced substances and the microorganisms themselves. It can be expected that novel fermented beverages will be developed that meet the consumers demand for tasty and healthy beverages.

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Glossary

Coculture	Defined mixture of different microorganisms/organisms, proliferated under sterile conditions.
Mixed culture	Undefined culture of different microorganisms/organisms, under unsterile conditions, spontaneous fermentation.
Pure culture	Defined, single culture, proliferated under sterile conditions.

4

NEW INSIGHTS ON WINEMAKING OF WHITE GRAPES

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Wines present a very complex chemical composition (Dalipi et al., 2015; Değirmenci Karataş et al., 2015). Especially, the aromatic character of dry white wines is of increased interest to researchers and consumers.

This chapter begins with the complex problem of grapes quality (first stage), continuing with the picking, transporting, cooling, reception, and the grape harvest treatment (second stage), the grape processing stage (third stage), the prefermentative stage, separation, and settling of the grape must (fourth stage), followed by the alcoholic fermentation (AF) of the must and the facultative malolactic fermentation (MLF) of the wine (fifth stage), the post-fermentation step of the new wine (sixth stage) followed by the wine maturation/aging stage (seventh stage), clarification and stabilization of wine (eighth stage), and followed by conclusions and final recommendations.

4.1 Quality of White Grape Varieties

The quality of the grape harvest depends on its degree of maturation which highlights its aromatic potential. This aromatic potential divides white grape varieties into two main groups, such as varieties rich in volatile monoterpenes and their precursors (Gambetta et al., 2014) accompanied by norizoprenoids (Muscat, Chardonnay, Rhein Riesling, Pinot Gris, Gewurztraminer, etc.) and varieties rich in volatile thiols and their precursors (Mateo-Vivaracho et al., 2010;

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Pena-Gallego et al., 2012) accompanied by methoxypyrazines (MPs) (Sauvignon blanc, Semillon, etc.). In each of these groups, other secondary metabolites with fewer odors are included. Based on aforementioned classification, the winemaking technology should also be differentiated.

In white wine production common analytical parameters (sugars, acidity, pH, phytosanitary status of grapes, etc.) and specific ones such as near-infrared spectroscopy (NIRS), berry tasting are used to establish the optimal time for grape harvesting (Nogales-Bueno et al., 2014; Deloire, 2014).

Reynolds and Balint (2014) revealed that volatile monoterpenol precursors content depends on viticultural and oenological practices. Free volatile monoterpenol and volatile monoterpenol precursors content are rarely correlated with sugar content, total acidity, or pH, and a decrease in free volatile monoterpenols and volatile monoterpenol precursors can occur between the berry and must stages, because of the skin contact (Olejar et al., 2015).

4.2 Harvesting, Cooling, Transport, Reception, and Treatment of Grape White

At this technological stage, the main objective is to protect the grapes harvest against oxidation on contact with oxygen from the air. The success of this stage is common to both categories of dry white wines with distinct sensory profiles mentioned above. Folliar treatments with addition of inactive dry yeast products as well as other preharvest treatments (Téllez et al., 2014), are able to provide effective antioxidant protection until grapes are processed.

Grapes harvesting can be done manually and mechanically, depending on the phytosanitary grapes status and on the technological destination of the harvest grapes. According to the odorant precursors, the use mechanical harvest grapes may induce a significant loss of 55% of the P-4MMP precursor and 30% of the P-3MH compared with manual harvest when the loss was lower (14% for P-4MMP and 8% for P-3MH) (Agosin, 2013).

Grapes cooling is needed to ensure a lower temperature (approximately 20°C) by using snow or carbon ice (Jaeger, 1996). The use of cooling agents, technique of administration together with the advantages of using CO_2 as snow or carbon ice are already detailed in the literature (Croitoru, 2008b, 2009; Ferrarini et al., 2006).

Grapes transport is provided in refrigerated tanks fitted with optimal cooling systems (at higher distances) or with other way of transport (at lower distances). It is very important to maintain a lower temperature of the grapes harvest during the transport to the cellar, taking into account the exterior high temperatures during the harvesting period (Capone and Jeffrey, 2011).

Grapes reception by using improved quantitative reception systems that automatically record the grapes quantities and allow the rapid and homogeneous sampling for qualitative reception are used.

Antioxidant protection treatments are provided with bicomponent products (based on potassium metabisulfite and ascorbic acid) at doses of 20 g/hL which release more than 50 mg/L of SO_2 which provide an antioxidant effect of ascorbic acid 10 times greater than its separate use or in combination with SO_2 (AEB-Spindal, 2013) or with glutathione at doses of 10–20 g/hL (Makhotkina et al., 2014).

4.3 Processing of White Grapes

Grape processing is made by innovative mechanical and optical methods in order to obtain the mixture of must and skins and to separate the stems. This stage involves destemming and separating the stems, grading the berries by eliminating the vegetal and mineral impurities, and finally crushing the berries. It is recommended to integrate the Delta Trio rotary sorting table, the Delta Rflow air jet separator, or the Delta Vistalys high precision optical sorter, in combination with the Oscyllis which is used for destemming. Also, Delta Elvtr and Delta Tre mobile elevators can be involved (Vaslin, 2012).

Grape destemming and stems separation ensure the separation of whole berries from their pedicel and rachis and to exclude the total stems vegetal mass. Destemming shall not affect the integrity of the stems or berries. Breakage, fragmentation, crushing, or shredding of the stems or skins affects the quality of the raw material. In the current white winemaking, the destemming operation is mandatory.

Applying the destemming following advantages: reduction of production capacity volume (tanks) and the volume of crushed berries for pressing; decreasing of herbaceous character (due to the presence of stems); increasing alcoholic degree and acidity (stems are rich in potassium and their presence decreases the acidity cannot be diminished); and decreasing the content of tough tannins (responsible for disagreeable vegetal and herbaceous characters) are provided (Vaslin, 2012).

According to the abovementioned advantages, the generalization of destemming induces some technological inconveniences in white winemaking, such as: complicating the pressing (the absence of stems which provide drainage channels) and the difficulty of performing AF (less air that would have been absorbed in the presence of stems) (Vaslin, 2012).

For white grapes destemming, the Oscillys 100 (produced by Bucher Vaslin) is recommended. It uses an ample swinging motion of the one or two cages to separate the grapes from the stems by inertia. It consists of a feed hopper, a swinging motion destemming system, a stems collecting system, and a berries separation and collecting system (Vaslin, 2012).

Berries sorting and impurities separation is applied only to healthy and mature raw white grapes used for the production high quality wines. The operation consists the removal of vegetal rests (leaves and stems) and moldy or insufficiently ripped berries. Berries sorting is only possible on grapes (or on berries) from manual harvesting and is not recommended for mechanized harvesting. It can be achieved at different technological stages: when transporting grapes immediately after harvesting (very rarely); on the sorting tables placed between reception and destemming operations (most frequently); and on sorting tables placed between destemming and crushing operations (less frequently) (Vaslin, 2015).

Many studies have highlighted the impact of some vegetal rests on the sensory profile of white wines. It was proved that only about 1% of the plant present in the must can give to final white wine a disagreeable taste of green; a percentage from 0.3% to 1% of the vegetable residues present in the must, will reduce the green odor intensity; only in the case of less than 0.3% of the vegetable residues present in the must will not affect the odor profile of the resulting white wine (Vaslin, 2015).

For berries sorting, different equipments may be used, such as: Delta E2, Delta Oscillys E2, Oscillys Trio, Rflow, and Vistalys (Vaslin, 2015).

Finally mobile elevators TRE and ELVTR have been used starting with Autumn 2015 (Vaslin, 2015).

Protected crushing of sorted berries (light berry crushing) is done in order to release the must, and to avoid the seeds breaking, the pulp destroying, and skins fragmentation. The resulting crushed berries have to contain the solid fraction (skins, pulp, and seeds) and liquids (must). The distance between the rollers must be adjusted accordingly to avoid the crushing of skin and the broking of seeds (Vaslin, 2012). The main disadvantage of crushing is the rapid saturation of fresh must oxygen immediately after its release from the vacuole within the first 5 min, so it requires effective antioxidant protection (Ribereau-Gayon et al., 2004). In modern winemaking, crushing is avoided, and whole berries are circulated directly into closed pneumatic presses (Vaslin, 2013).

Operations required before pressing (especially before pressing under inert gas) are to reduce the oxygen action on musts and to limit the harvest degradation, to avoid the release of the must prior to pressing and the oxygen intake during the transfer phase. In this context, it is indispensable to prepare harvesting, transport, and processing circuit that is effective and involve: the use of a properly adjusted harvesting machine; grape carrying during in order to avoid high temperatures and to slow down oxidation processes; using a vibrating bunker (more useful than a snail bunker) for harvested grapes; using the gravity transfer of the grapes with a helical pump or with a peristaltic pump (in the case of mechanical harvesting); and using the destemmer that does not affect the grapes and the stems structure (which must be kept them as inaccessible as possible) (Vaslin, 2013).

4.4 The Prefermentative Step, Separation, and Settling of the Must

This stage includes pellicular maceration and enzyme treatment of musts, antioxidant protection of musts together with berries pressing to obtain must fractions during a full cycle of pressing, influence of grape processing techniques on sensory properties of dry white wines, and clarification of free run and press musts. Also, some particularities of the prefermentative stage are approached for varieties rich in volatile monoterpenols and volatile thiols and their precursors. The problem of cooling will be addressed separately as a particular element for both categories of dry white wines with distinct sensory profiles.

4.4.1 Pellicular Maceration vs Enzymatic Treatment of Crushed Berries

More and more oenologists avoid to use the cold prefermentative maceration under reductive conditions (Di Lecce et al., 2013) and the enzymatic treatment (Canal-Llauberes and Nedjma, 2015). Between the two technological solutions, there are convergent but mostly divergent elements as listed below.

Both treatments purpose is to extract odorants and their precursors from berry skins (free and bound volatile monoterpenols in precursors, volatile thiols and cysteinyl and glutathionyl precursors, glycosylated C13-norisoprenoids, MP, etc.), by cold natural enzymatic maceration or by treatment with exogenous enzyme preparations under optimal technological conditions. Important is to achieve an optimal compromise between maximal extraction of odorant compounds and their precursors and minimal extraction of cinnamic acids and other phenolic compounds with disagreeable sensory character (Olejar et al., 2016).

The extraction of odorant compounds and their precursors from destemming and crushed berries may be accomplished by slow pressing in closed pneumatic presses. It is successfully applied to the Sauvignon, Semillon, and Muscadelle varieties, but also to the Muscat, Chardonnay, or Riesling varieties. Closed pneumatic presses are ideal for applying enzyme treatments to crushed berries (Vaslin, 2013).

Particularities of cold prefermentative maceration refer to avoid the must oxidation, to establish certain technological and analytical parameters.

To avoid oxidation, equipments under CO_2 atmosphere can be provided. The most interesting variants are the following: closed pneumatic presses; recipients that assure the gravity transfer of crushed berries; membrane presses (Elite-Péra type) which are a hybrid between a pneumatic press and one in tank; and rotative tanks (Ribereau-Gayon et al., 2004).

The duration (from 4 to 6 h to 20–24 h) has to be correlated with the temperature (optimal temperature is 8–11°C and the maximum allowed is 15°C); the crushed berries cooling in CO_2 atmosphere in the container allow the "hunting of oxygen" and providing the antioxidant protection. The advantage of maximum efficiency is balanced by a very high cost. The evolution of some analytical parameters includes the acidity decrease by 1.5–2.2 g/L in tartaric acid (simultaneous with a pH increase) due to the release of potassium from the skin and the salification of the tartaric acid, the slight increase of OD_{280nm} which does not affect quality (remains below 10), but also the increase in amino acids, proteins, and neutral polysaccharides, so that the resulting dry white wines will require higher doses of bentonite for their protein stabilization. The cold prefermentative maceration of crushed berries has a limited character because it is appropriate only for healthy white grape varieties with an advanced stage of maturity (Ribereau-Gayon et al., 2004).

Particularities of enzymatic treatment of crushed berries. This treatment is applied in two to three stages as a liquid suspension by solubilizing the enzyme preparation, directly in the must (5 g/hL, at temperature of 15°C), during the filling of closed pneumatic press. At temperatures below 15°C and in the case of application to contaminated crushed berries, the efficacy of enzymatic treatment becomes uncertain. Researches on the criteria for choosing enzymatic preparations according to the main, secondary, and special activities, their classification according to the technological destination, factors influencing the enzymatic treatment but also the monitoring and performance of enzymatic treatment are already reported (Croitoru, 2009; Canal-Llauberes and Nedjma, 2015).

Enzymatic treatment has been proven to be effective in less maturated healthy grapes (with lower concentrations of free varietal aroma and higher concentrations in their precursors). The effectiveness of extraction treatment of free varietal aroma and especially of their precursors from white grape skins is decisive because these precursors are found in concentrations three to five times higher than free aroma (Canal-Llauberes and Nedjma, 2015).

4.4.2 Antioxidant Protection and Mash Pressing

Classical antioxidant protection is done by addition of sulfur dioxide or products based on sulfur dioxide and ascorbic acid. Nowadays, antioxidant protection during pressing under inert gas is increasingly promoted (Vaslin, 2013). Other researches are focused on developing technology solutions to increase glutathione content (GSH) from mash which is a very powerful natural antioxidant (Šuklje et al., 2016; Gabrielli et al., 2017).

Inert gas protection during the pressing cycle. When dry white wines are produced, shortening the flow to avoid oxidation requires the direct filling of chilled mash in the pneumatic press, eliminating the cold prefermentative maceration. It is recommended to use the Inertys process which involves the filling of the pneumatic press with mash into the inert gas atmosphere which carries out the full pressure cycle under inert gas protection (Vaslin, 2013).

In general, the use of sulfur dioxide for grapes or mash ready to be pressed must be done at very low and limited doses only in some technological situations. The presence of sulfur dioxide has the major effect to "perforate" the cell walls of berry skins, thus facilitating the extraction of polyphenols and especially of catechins (Corona, 2010).

Other researches (Dubernet, 1974) and later (Nikolantonaki, 2010) tend to minimize the antioxidative activity of SO_2. At the dosages currently used to fill the presses, there is only an inhibition of enzymatic activity, but never an instantaneous stopping of it.

In the process of making dry white wines, the pressing operation fulfills several functions: the extraction of must from the mash, favoring the diffusion in this must of certain skin compounds, such as free varietal aroma expressing fruitiness, but especially their precursors found in higher concentrations; limiting the passage into the must of bitter-tasting compounds and phenolic compounds from the mash that can damage the aromatic stability of the future wine (Vaslin, 2013).

Physical actions on the mash during pressing will influence the quality of the resultant must fractions (Boselli et al., 2010).

Musts from the end of the pressing cycle give less flavored wines because to the oxidation of polyphenols and to the formation of the herbaceous taste during the pressing. Although the presence of polyphenols is recognized for their appreciated taste (body, volume, and bitterness), their oxidation is one of the causes of these varietal flavor losses. Dissolving the oxygen is very fast in the must during prefermentative operations and induces oxidations that modify the phenolic composition of the must (Ribereau-Gayon et al., 2004).

Musts' browning occurs at the end of the pressing cycle and is the most obvious consequence of the musts oxidation. This reaction is caused by the polyphenols oxidation in quinones involving an enzyme (polyphenoloxidase). The absence of typical aroma in table wine is not only associated with the loss of an important content of varietal volatile thiols, but also with other phenomena. Besides the oxidation of polyphenols, other enzymatic reactions occur during the pressing (e.g., oxidation of fatty acids). This oxidation involves the formation of 6-carbon compounds (C6) which contribute to the vegetal odor in wine (Ribereau-Gayon et al., 2004).

Mash oxidation and polyphenol extraction are main parameters with high impact on the quality of final white dry wines (Ribereau-Gayon et al., 2004).

The filling phase of the press has an inevitable impact on the quality of the extracted must due to the risks of destroying the berries skins and, consequently, the extraction of polyphenols. Some rules of good practice have to be respected. These rules refer to the degree and mode of filling the press. Choosing inert gas (CO_2 or N_2) can have some influence on the quality of the final wine. The carbon dioxide has the particularity of dissolving in the must, so it can limit oxygen absorption. Hence, it provides a better protection of the must against oxygen, but its consumption is quite significant (Vaslin, 2013).

The only way to stop the action of oxygen is to inject the inert gas during the prefermentative stage, but especially during the pressing operation. This operation requires the management of different flows (must flow and gas flow) and the management of the various aggregation states reported in the pressing process (mash solid phase, must as liquid phase and inert gas as gaseous phase) (Vaslin, 2013).

The Bucher Inertys process is a patent recognized today worldwide and has been validated in several national and international technical centers. The composition of the must is different during pressing: the first fractions of extracted must are provided from the pulp followed by those extracted from the skin. Depending on the nature of the variety and the viticultural area, the phenolic composition of the must from the skin may be very different from that of the pulp (Vaslin, 2013).

During pressurization under inert gas, a rigorous control of the phenolic richness of the musts from the last pressures is recommended. Musts provided from the last pressures (from pressures greater than 1.5 bars) are richer in catechins and epicatechines, so they represent a major risk for the aromatic stability of the future wine (Nikolantonaki, 2010). For this reason, it is recommended to separate the last fraction of must especially for those varieties rich in polyphenols.

The last must fraction will have to be treated separately for the purpose of reducing its phenolic composition. The amount of must from the last pressing is 5%–10% of the total volume of extracted must. Fig. 4.1 shows the very intense color of the must resulting from the

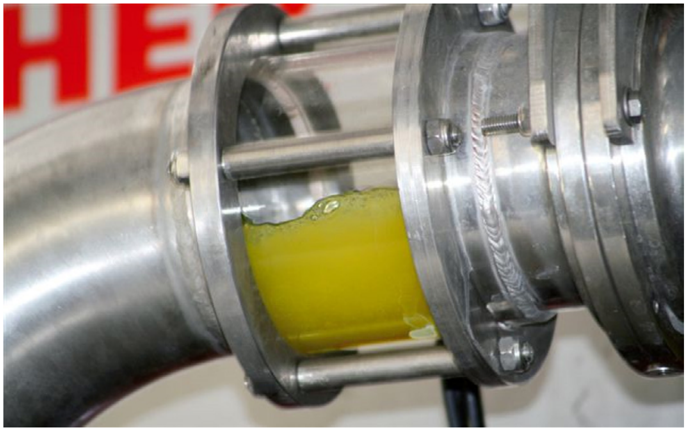

Fig. 4.1 Must resulted from pressing with the Bucher Inertys equipment (Vaslin, 2013).

pressing with the Bucher Inertys equipment, and in Fig. 4.2, the pronounced greenish chromatic color of the pressed marc. These figures demonstrate that this equipment allows the complete avoidance of contact between oxygen and grape or must during the entire pressing process. An experiment above on the color of Sauvignon blanc wines shows the difference between the wine from the must obtained with the classic pneumatic presser XPF 80 (brown red) and that one obtained with a Bucher Inertys (green green) press is shown (Fig. 4.3) (Vaslin, 2013).

Natural protection of the mash by increasing concentration in glutathione. In white winemaking, it is important to establish the minimum/optimal amounts of free sulfur dioxide and/or ascorbic acid

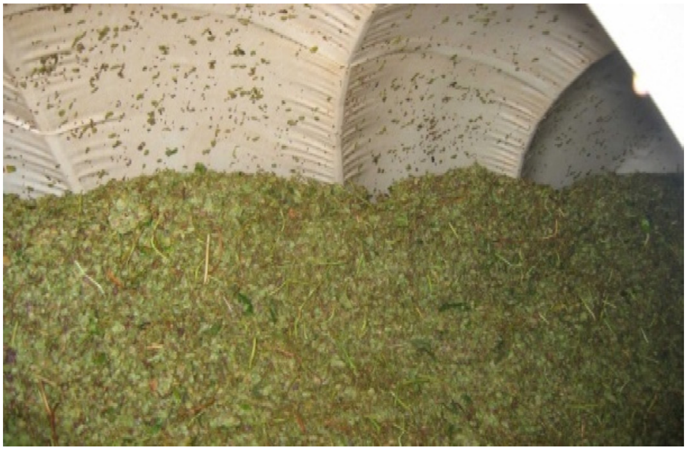

Fig. 4.2 Pressed marc after pressing by Bucher Inertys equipment (Vaslin, 2013).

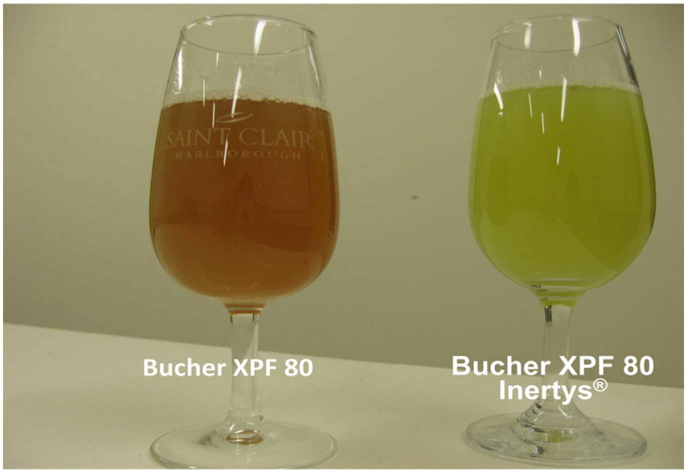

Fig. 4.3 The visible color difference of the must after pressing by using a Bucher pneumatic press and a Bucher Inertys pneumatic press (Vaslin, 2013).

needed besides GSH to assure the wine stability (Badea and Antoce, 2015). According to both OIV (International Organization of Vine and Wine) resolutions (OENO 445-2015 and OENO 446-2015) were adopted. They define the use of glutathione (GSH) up to a maximum level of 20 mg/L in must and wine (Wegmann-Herra et al., 2016).

Using GSH in winemaking prevented the oxidative browning of musts (Hosry et al., 2009) and its antioxidant ability can be an evaluation criterion (Sarakbi and Kauffmann, 2014), but no predictable benefits on sensory characteristics of young Riesling, Chardonnay and Sauvignon blanc wines were observed (Wegmann-Herra et al., 2016).

The addition of pure glutathione (GSH) is not allowed under current OIV regulations but the concentration of this compound can be increased in wine through the addition of glutathione-enriched dry yeast preparations (DYP). They have antioxidant properties and could influence wine aroma and sensory characteristics of Sauvignon blanc Gabrielli et al. (2017) reported that the addition of DYP preparations to must increase the concentration of some wine volatile compounds, with increased attributes of riper tropical fruit aromas, which was not always observed with the GSH additions.

4.4.3 Influence of Grape Processing on the Sensorial Properties of Dry White Wines

Usually, white wines are classified on the basis of their flavor intensity, acidity, and sweetness, but also according to the mouthfeel attributes. These differences in white wine mouthfeel have been attributed to phenolics (Patel et al., 2010; Cejudo-Bastante et al., 2011), glycerol (Jones et al., 2008), ethanol (Gawel et al., 2007; Nurgle and Pickering, 2005), sugar (Nurgel and Pickering, 2005), and polysaccharides presence (Jones et al., 2008; Vidal et al., 2004).

The majority of white wine is made from must that is obtained by draining crushed grapes under the action of gravity immediately following destemming and crushing (Gawel et al., 2014). When a wine with a smoothness mouthfeel (i.e., a fuller bodied style) is desired, winemakers may allow grape skins to stay in contact with juice (Maggu et al., 2007) prior to draining (skin contact) or they may include the juice pressed off the wet skins that remain after draining (pressings), as both methods result in more flavor some wines with higher phenolic concentration (Darias-Martínet et al., 2004).

Musts with lower phenolic concentrations that are destined for the production of less intensely flavored wines with a “thinner” mouthfeel (i.e., lighter bodied styles) can be obtained by applying pressure to whole uncrushed bunches (whole bunch pressing).

There is also a significant difference in phenol extraction between different grape processing methods (Patel et al., 2010) for white winemaking: crushing before pressing releases ~40% of grape phenols compared with ~18% for whole-bunch pressing without crushing. Understanding how extraction of phenols from grapes changes as a function of fruit-processing and winemaking practices may aid in mitigating and managing smoke taint in smoke-exposed grapes (Kelly et al., 2014).

A prolonged skin contact period may contribute to increased wine complexity, with the appearance of interesting new sensory attributes (Ferreira-Lima et al., 2016). Moreover, changes in mouthfeel properties, such as astringency, sourness, and bitterness, are to be expected due to changes in wine composition. Finally, an increased wine aging potential might have been achieved, which also needs further investigation (Aleixandre-Tudo et al., 2015).

The ability to influence wine composition by applying different juice extraction and handling methods partly arises from differences in the relative concentrations of phenolic types found in the pulp, skins, and seeds of the grape (Gawel et al., 2014).

Pulp cell vacuoles contain relatively low concentrations of phenolics compared to skins and seeds and are mostly represented by hydroxycinnamic acids, whereas seeds contain significant amounts of flavanols and skins contain flavanols, flavononols, flavonols, and hydroxycinnamic acids (Di Lecce et al., 2014).

4.4.4 Antioxidant Protection of the Must Fractions After Mash Pressing

These operations refer to the sulfitation of the must and the need to reduce the content of polyphenols in the last pressing must to both the conventional pressing and the inert gas protected pressing process.

The extracted musts have to be sulfitated as soon as possible after the ending of the pressing cycle with a dose that allows inhibition of fermentative activity and antioxidase activity in the presence of inert gas protection. Otherwise, glutathione degradation and oxidation mechanisms start as a consequence of oxygen dissolution in the air during the pumping and degrading phases of the must, as well as other operations in the prefermentative phase (Du Toit et al., 2007).

Technological solutions to reduce polyphenols content in press must are as follows:

- Addition of polyvinylpolypyrrolidone (PVPP) is effective, but the adsorption time of polyphenols on PVPP particles is often too long (several days) (in the case of a must resulted from pressing with or without inert gas) (Roman et al., 2016).
- A controlled oxidation of the polyphenols which causes precipitation (in the case of a non-sulfitated must resulting from inert gas pressing). The last process is efficient because it gives the resulting wine an extra body and a lasting aromatic stability (Du Toit et al., 2007).
- By using nonconventional methods, as high-pressures treatment on the inactivation of polyphenol oxidase from grape must (Râpeanu, 2008).

4.4.5 Settling of Free Run and Press Musts

The collected must fractions (free run and press) can be settled either by classical methods (refrigeration, sulfur dioxide treatment, bentonite treatment, centrifugation, etc.) or by biotechnological treatments (exogenous enzymatic preparation addition that accelerates the formation of suspension and sedimentation at the bottom of the vessel) or by combined techniques (by flotation associated with enzymatic treatment and clearing agents).

Biotechnological clarification processes. It is the static clearing of the must by enzymatic treatment. Understanding the mechanism of static clarification highlights better the opportunity for enzyme treatment. The viscosity of musts is due the presence of pectin substances (pectin) which forms a gel that blocks the suspension that induces a very slow spontaneous flocculation of them. Under these conditions, electrostatic interactions necessary for must's clarification are very difficult to establish (Canal-Llauberes and Nedjma, 2015).

Advantages of enzymatic preparation addition for static clarification refer to: hydrolysis of soluble pectin by the action of pectinlyase, pectinesterase, and polygalacturonase activities; fast decrease of must viscosity; fast must clarification limiting its oxidation risk; no release of cinnamic acids, potential sources for unwanted compounds (Canal-Llauberes and Nedjma, 2015).

Combined clarification processes. This category includes clarification of musts by flotation accompanied by enzymatic treatment. Must clarification by flotation technique becomes increasingly in white winemaking. Clarification of must in floators is achieved by flotation phenomenon that separates the clarified must at the top and the suspensions under the action of injected fining agents along with the drawn air at the bottom. During flotation, the addition of some enzymatic preparations is recommended with a period of action of 1–4 h at 15–18°C in the presence of gelatine, bentonite, silica gel, and airflow (Croitoru, 2012).

4.4.6 Prefermentative Operations With Impact on White Grapes Rich in Volatile Monoterpenols and Their Precursors

The impact of technological prefermentative operations prior to this technological stage is concerned on volatile monoterpenols and their precursors of white grape varieties and then to other categories of odorous compounds that accompany them.

Taking into consideration the *influence of harvest date*, tasters could distinguish between wines from "early" and "late"—harvested fruit in five of six cultivars based on aroma, and three of the six based on flavor. In many cases, these tasters indicated that the late-harvested treatments had either the strongest Muscat and/or strongest floral character (Reynolds and Balint, 2014).

Skin contact seems controversial among some winemakers. Some of them have indicated that use of and duration of skin contact can appreciably increase the concentration of specific terpenes in must and wine (Reynolds et al., 1993). Reynolds and Balint (2014) reported that there was no clear indication of whether skin contact resulted in more Muscat or floral character in the aroma or flavor.

Addition of the *enzymatic preparation* according to the values of the main enzymatic activities [pectin lyase (PL), polygalacturonase (PG), and pectin methylesterase (PME)] and secondary (arabanase, cellulase, etc.) take into consideration the maximum extraction of the free volatile monoterpenols and their precursors, but also of the C13-norisoprenoids simultaneously with minimal extraction of polyphenolic constituents (hydroxycinnamic acids); the used doses are 5–6 g/hL of mash, and the treatment is applied at a temperature of 16–17°C (Canal-Llauberes and Nedjma, 2015).

If this enzymatic treatment does not remove sufficiently high content of monoterpenols precursors from the skin, it will not be possible to create a "working front" for the second enzyme treatment; applied in the postfermentation phase, this treatment will release the volatile monoterpenols from their precursors (Croitoru, 2008b). The enzymatic preparation chosen for the extraction of volatile monoterpenols and their precursors also contributes to accelerate the must clarification resulting from the enzymatic treated mash (Canal-Llauberes and Nedjma, 2015).

The content in free volatile monoterpenols and their precursors decreased from the berry to the must stage. The decrease in free volatile monoterpenols was 52%, 41%, and 22% for Muscat Ottonel, Gewurztraminer, and Kerner, respectively, and their precursors were 16%, 52%, 13%, and 28% for Muller-Thurgau, Muscat Ottonel, Gewurztraminer, and Kerner, respectively (Reynolds and Balint, 2014).

4.4.7 Prefermentative Operations With Impact on White Grape Rich in Volatile Thiols and Their Precursors

In the case of white grape varieties rich in volatile thiols and in their precursors, some prefermentative operations or anterior technological stages significantly influence both the content of MP and volatile thiols.

Influence on volatile thiols. The prefermentation stage is the most important regarding the massive loss of thiol precursors, evaluated for three wineries and three clones of Sauvignon blanc grapes; the decrease vary from 45% to 92% for P-4MMP and 40% to 78% for P-3MH, while for the fermentation stage itself, the results are less significant (Agosin, 2013).

Influence on MPs. Limited skin contact and exclusion of stems or leaves from the winemaking process can reduce the amount of MP extracted into the resultant wines. Lighter pressing regimes can also reduce MP concentrations (Reynolds and Balint, 2014). Press wines contain higher concentrations of MP compared to free run wines (Roujou de Boubée et al., 2002).

Influence of GSH or DYP addition. The addition of GSH or DYP to Sauvignon blanc grape must has been shown to increase the concentrations of certain volatile compounds, which included thiols and some monoterpenes in the case of the DYP-added treatments. The amount of GSH released into the wines when DYP was added, however, seems to be limited, and the increases in GSH, volatile thiols, and other compounds are probably due to a change in the chemical composition of the must (Téllez et al., 2014; Gabrielli et al., 2017).

Enzymatic treatment of pomace. The use of the enzymatic preparation has a decisive influence because the potential odor of the must consists of 5%–10% free volatile thiols and 90%–95% cysteinylated and glutathiolated precursors and together with the other specific aromatic constituents (Coetzée and Du Toit, 2012). The doses may vary between 3 and 6 g/hL of pomace, and the treatment is applied at temperatures not exceeding 18°C (Roland et al., 2010).

Enzymatic treatment of the must. A complex enzymatic preparation with many enzymatic activities (pectinases and proteases), but the most interesting is carbon-sulfur-lysate which hydrolyzes the aromatic thiol precursors of must (nonvolatile and odorless) in their thiols (volatile and odorants) has been developed and tested. Doses from 3 to 5 g/hL are recommended. The addition is recommended to be after 3 days of fermentation directly to the must, but in the final wine (minimum temperature is 15°C) as well. The OIV allows the use of this type of enzymatic preparation according to the latest regulations recently added to the EU Commission Regulation no. 53/2011.

Cold storage of clear must. Before the beginning of AF, cold storage of clear must (about 5°C) is recommended, this may vary from a few days to several weeks. The phenomena that occur during this storage are surprising, and the lees of the must remaining in the must present a favorable role increasing the concentration in volatile thiol precursors (cysteinylated and glutathionylated precursors) (Roland et al., 2010). The reaction mechanisms of these precursors increase are not fully known. It is possible either a modification of the must composition which results in a stronger conversion of the precursors into their corresponding thiols during the must AF, or the formation of another precursor to be identified.

Additional enzymatic treatment. Enzyme treatment of cold stored must with a superconcentrated enzymatic preparation suitable for a restrictive thermal regime (5–8°C) used at optimal doses (3–5 g/hL) contributes significantly to the increase the volatile flavor precursors of must. Inert gas protection during this cold storage is mandatory in order to decrease the formation of quinones with high reactivity to glutathione (AEB-Spindal, 2013).

Quinones and their influence. Quinones are true barriers to varietal flavor compounds (Nikolantonaki, 2010). This reaction mechanism of quinones (oxidized flavanols) with volatile thiols liberated under the action of the yeast strain during AF explains in part the differences observed between wines obtained from free run musts and ones from end pressing cycle. This explanation highlights the fact that a part of the oxidized polyphenols (quinone) in the prefermentative stage remains in the must and does not change its state. The AF process which take place in a reducing medium, does not affect the state of the formed quinones, and for this reason, these quinones retain the ability to fix the volatile thiols released from their precursors during the fermentative process under the action of the yeast strain.

Clarifying agents and 3-sulfanylhexanol precursors in grape must. *S*-3-(hexan-1-ol)-l-cysteine (Cys-3SH) and S-3-(hexan-1-ol)-l-glutathione (GSH-3SH) are precursors, present in grapes and/or in must (Tominaga and Dubourdieu, 2000; Peyrot des Gachons et al., 2002; Schneider et al., 2006; Fedrizzi et al., 2009).

The grape variety, as well as the processing conditions of grape, pomace, and juice, is very important in order to obtain a high level of precursors (Roland et al., 2011; Cerreti et al., 2015; Román Villegas et al. 2016). Cys-3SH precursors increase with longer skin-contact and stronger pressing conditions (Mattivi et al., 2012) while the GSH-3SH in particular increases when oxidative prefermentative maceration is adopted (Larcher et al., 2013).

Roman et al. (2016) who studied the musts treated with Na-bentonite, PVPP or charcoal (1 g/L) and cold settled found that the clarifiers used in must affected the thiol precursors in a marginal manner (only charcoal was able to significantly reduce 3-sulfanylhexanol precursors in must).

4.5 Fermentation Stage: AF of Must and Optional MLF of Wine

4.5.1 General, Innovative, and Specific Elements of the Must AF in White Winemaking

General elements of the must AF in white winemaking. The general elements are relating to choose the selected *Saccharomyces* yeast strain (Coulon and Renouf, 2015), the right nutrients, the stages of intervention with these technological auxiliaries, controlling the performance of the AF and monitoring its thermal regime, as well as other AF common elements of the musts with implications on the sensory profile of the resulting wines.

The yeast straines. AF is carried out by yeast, mainly the more alcohol tolerant *Saccharomyces cerevisiae* (Coulon and Renouf, 2015), that convert sugar to ethanol and CO_2 as principal products and secondary products (glycerol, superior alcohols, aldehydes, etc.).

The final aroma profile of wine is direct connected to the selected yeast strain involved in fermentation because of the secondary fermentation products such as esters, higher alcohols, aldehydes, and fatty acids (Styger et al., 2011).

Nowadays, the yeast strain selection is based on DNA fingerprinting methods [MLVA (multiple-locus variable number tandem repeat analysis) method, polymerase chain reaction (PCR) method, and capillary electrophoresis method] to discriminate between closely related strains of indigenous and commercial *S. cerevisiae* (Coulon and Renouf, 2015).

Descenzo (2016) reported that although vineyard strains can be recovered from non-inoculated winery fermentations, the fermentations appear to be conducted by yeast strains resident in the winery, which seem to be a mix of commercial strains used in the winery as well as noncommercial strains.

The yeasts nutrients. Phosphate, $(NH_4)_2HPO_4$ and diammonium sulfate $(NH_4)_2SO_4$ are the ammonium salts used in oenology at a maximum dose of 100 g/hL (which correspond to 210 mg/L of easy assimilable nitrogen). To choose of the correct dose requires attention because only the addition of 10 g/hL of ammonium salts can increase the acidity by 0.5 g/L in tartaric acid (Ehsani, 2015). The association of ammoniacal nitrogen with thiamine is indispensable (Ehsani, 2015). Thiamine in must ranges from 160 to 450 μg/L (Ribereau-Gayon et al., 2012) and can be used only few hours after the starting of AF (Bataillon et al. 1996) by selected *S. cerevisiae* strains able to consume amounts from 600 to 800 μg/L (Ehsani, 2015).

An addition of 50 mg/hL thiamine significantly reduces the content of certain compounds (pyruvic and α-ketoglutaric acids) that rapidly combine with SO_2 (Ribereau-Gayon et al., 2012). However, complex nutrients based on yeast derivatives (inactive cells, autolyzed, cells coatings, etc.) ensure that nutritional deficiencies and regular kinetics are avoided by exerting favorable stimulating and detoxifying effects on the fermentative medium (Ehsani, 2015).

The stages of intervention. The starting of AF refers to the administration of selected *S. cerevisiae* strains at doses of 10–20 g/hL and simple nutrients at doses of 10–15 g/hL (Coulon and Renouf, 2015). It is recommended that the first yeast strain to be selected from a representative vineyard in order to guarantee the typicity of the wine. The oxygen required for yeast cell growth during AF should be given after 3 days of the AF starting, which usually coincides with a 1/3 reduction in the must density, when the conditions offered by the fermentative medium become favorable: the number of yeast population is high enough to consume rapidly the oxygen; the fermenting medium has been lowered in oxygen due to the AF process; the activity of oxidation enzymes is reduced (Ribereau-Gayon et al., 2012).

The final stage of AF is reached when the density of the fermentative medium decreases to 1030–1040 g/L (*Saccharomyces bayanus* strains at doses of 10 g/hL and complex nutrients at doses of 20–30 g/hL should be used) (Ehsani, 2015).

Controlling the fermentative process. In white winemaking, the control consists in managing fermentation tanks (by filling the vessels), fermentation kinetics (by daily control of temperature, density, alcohol concentration, and yeast cell density) and the oxygen content of the must (with an essential role in the synthesis of sterols and long-chain unsaturated fatty acids acting as survival factors) (Ribereau-Gayon et al., 2004).

Managing the temperature. To control the temperature in tanks (barrels, stainless steel tanks, etc.), the correlation between the temperature and the concentrations of secondary products in AF, to avoid thermal shocks, the influence of the fermentation tanks geometry, but also the technical features (cooling devices).

Finding the end of the fermentative process. It is determined by the AF duration (it must not be longer than 12–14 days if it has been correctly managed and controlled), the value of the must density during AF (when it decreases below 0.994–0.993 g/L). The operations performed immediately after the end of AF consist in filling of the tanks, decreasing the temperature progressively to 12°C, protection against oxidation, and sulfitation of young wine at doses of 4–5 g/hL SO_2 after 1–2 weeks after AF ending (Ribereau-Gayon et al., 2004).

Del Bario-Galan et al. (2012) reported that the presence of neutral polysaccharides from yeasts is important for some sensorial characteristics of wines such as: decreasing the acidity and the optical density at 420 nm, increasing the softness on the palate.

Casalta et al. (2013) highlighted the interaction between grape solids and assimilable nitrogen content pointing out the importance of taking into account the balance between assimilable nitrogen and lipids for controlling fermentations during white winemaking.

Innovative elements AF of white wine grape musts. The innovative Wininq system for AF monitoring, AF realization by the use of immobilized selected yeast cells, but also monitoring the AF's thermal regime with new prototypes of more efficient installations were included (Neves et al., 2014).

In order to identify a wine from a given region environmental factors, including climate, geography, and soil have to be taken into consideration (Hénaff et al., 2016).

A correlation between regional microbial strains and differential wine phenotypes was reported (Knight et al., 2015). Due to their metabolic properties, microbial strains are responsible for many of the organoleptic characteristics of wine (Belda et al., 2016).

During the winemaking process, many factors may affect the winemaker's decision which can modify the expression of the terroir in the final product. WineSeq is a methodology to identify the relevant microbial communities throughout the winemaking process from soil to bottle, and the data science used to interpret the results (Hénaff et al., 2016).

The main aim of the WineSeq project is to characterize the microbiome of different wine regions around the world by studying the microbial composition of vineyard soils. By applying the next-generation sequencing (NGS) technology, an intelligent platform for analyzing and interpreting metagenomic information from an oenological point of view may be developed (Hénaff et al., 2016).

AF based on selected immobilized yeast cells. *S. cerevisiae* yeasts can be immobilized by natural adsorption on different supports. Grape skins were found to be an appropriate long-term use support for *S. cerevisiae* immobilization to carry out the AF in a winemaking process. The immobilized yeasts could be stored at least 1 month, at 4°C, without losing its biological activity and operational stability (Genisheva et al., 2014).

Monitoring AF's temperature with new prototypes of more efficient equipments. Neves et al. (2014) describe a low-cost prototype to perform the supervision and control of the AF process in a winery. The solution is modular and the experimental test performed to show the viability of the designed solution, allowed the researchers to identify possible improvements to optimize the system and make it more robust.

Specific elements of must AF white winemaking. AF included the use of selected non-*Saccharomyces* yeast strains and simultaneous or sequential AF by co-inoculation of selected non-*Saccharomyces* and *Saccharomyces* yeast strains.

AF with selected non-Saccharomyces yeast strains. In order to select an yeast strain with appropriate enological qualities and adapted to the ecological environment of the cellar to be used as starter culture, a number of 240 *Saccharomyces* spp were isolates and then characterized by DNA mitochondrial restriction analysis and finally the enological characteristics, nitrogen requirements, protein haze stabilization, and enzymatic activities and microvinifications were performed (Ortiz et al., 2013). Diaz et al. (2013) characterized the wild yeasts in the winery environment by using a PCR-restriction fragment length polymorphism (RFLP) method. They observed that *Metschnikowia pulcherrima*, *Rhodotorula mucilaginosa*, *Pichia kluyveri*, *Pichia membranifaciens*, and *S. cerevisiae* yeasts remained active at the end of the fermentation. High levels of acetic acid accumulated only in those fermentations with an extended lag phase.

Mixed AF by co-inoculation with selected non-Saccharomyces. Among these, the most interesting methods are those based on co-inoculation of 1–3 strains in simultaneous and sequential system, which can reduce the alcohol content of wines by the metabolic pathway (Coulon and Renouf, 2015).

In the last time, the use of mixed fermentation with *Saccharomyces* and *non-Saccharomyces* yeasts was tested (modalities of inoculum, temperature of fermentation, different grape juice) and then recommended because of the increasing content of 2-phenylethanol and glycerol and in term of tasting level because of the increase of spicy notes (Gobbi et al., 2013). Jolly et al. (2013) reported that the management of "mixed ferments" is more complex. The use of native apiculate yeast strains and cofermentation applications will contribute to better understand how to modify the "flavor phenotype" of wines and advance in the development of new yeast technologies (Medina et al., 2013).

In a study reported by Contreras et al. (2014), the use of non-*Saccharomyces* yeast strains resulted in a reduction of alcohol levels in wine by sugar respiration, confirmed also by Quirós et al. (2014). The use of mixed fermentation by *S. cerevisiae* and strains of *Candida zemplinina* or *M. pulcherrima* resulted in a decrease up to 0.9° for *C. zemplinina* and up to 1.6° for *M. pulcherrima* (Bely et al., 2013).

4.5.2 AF Particularities of Must Rich in Monoterpenols With Their Precursors

Except for the Muscat varieties, the Chardonnay variety is representative in this category, together with Rhin Rieslig, Pinot Griş, and even Gewurztraminer, along with other white varieties.

Techniques such as skin maceration, barrel, and stainless steel fermentation, use of selected or indigenous yeasts, MLF, and aging in barrels with or without lees are all applicable and lead to different compositional outcomes (Gambetta et al., 2014).

Selection of yeast strain follows the choice of a yeast strain specific to the white variety from which the must comes, but which, by its metabolic and fermentative properties, can enrich the sensory profile of the resulting wine (Croitoru, 2008b).

Yeast strains able to produce glycerol or to release mannoproteins but which also a reduced proportion of volatile monoterpenols from their precursors by the action of the β-glycosidase can be chosen in this case the thermal regime is maintained in the range of 15–20°C, when it is possible to obtain a more body wine accompanied by a pleasant fruitful—floral character, associated with medium freshness (Codreşi et al., 2012).

Yeast strains able to produce higher concentrations of pleasantly odorous esters and acetals and also generates organic acids. In this case, the thermal regime is maintained in the range of 13–18°C and the final wine is produced with a more intense fruitful floral character, associated with a more expressive freshness (Coulon and Renouf, 2015).

The application of a selected strain of *Hanseniaspora vineae* as an inoculum starter, in a co-fermentation, was successfully used for the production of quality white barrel fermented Chardonnay wines (Medina et al., 2013).

By using metabolic profiling study, the main metabolites involved in low-temperature response in three different yeast species from the genus *Saccharomyces* have been detected (Lopez-Malo et al., 2013).

Chalier et al. (2007) observed mannoproteins ranging from 5 to 100 kDa, and concluded that differences between yeast strains had consequences in aroma quality of wines and that the mannose composition of the mannoproteins secreted by *S. cerevisiae* strains.

4.5.3 AF Particularities of Must Rich in Volatile Thiols and Their Precursors

The presence of varietal thiols is not unique to Sauvignon blanc grape variety and can be found in many other white varieties such as Riesling, Colombard, and Semillon (Roland et al., 2011; Coetzée and Toit, 2012).

MP are highly extractable in wines (Roujou de Boubée et al., 2002) and very stable compounds due to their chemical nature and therefore are very difficult to remove or reduce in wines (Reynolds and Balint, 2014).

The use of oak barrels or oak chips during AF can reduce vegetal/herbaceous aromas/flavors in wines with high MP concentrations by a straight masking effect (Pickering et al., 2004, 2006).

The selected yeast strain to contain in its cells two indispensable enzymes, carbonsurfurlyase and alcohol acetyltransferase, which converts cysteinylated precursors into free varietal flavors typical of Sauvignon blanc (Swi et al., 2009). Carbonsurfurlyase transforms the cysteine-3-MMP compound into 4-MMP (4-methyl-4-mercaptopentan-2-one) identified by green and cranberry flavor (Howel et al., 2005; Swiegers et al., 2006) and respectively cysteine-3-MH compound in 3-MH (3-mercaptohexanol) identified by passion fruit and grapefruit flavors). Alcohol acetyltransferase converts 3-MHinto 3-MHA (3-mercaptohexanol acetate) identified by alcohol-like flavor (Swiegers et al., 2005b) (Figure 4.4).

Recent studies (Curtin et al., 2008) have shown that the release, conversion, and sensory perception of volatile thiols follow a much more complex sequential biochemical network by which 3-MH and 3-MHA compounds are formed. For these reasons, a deeper biological study is required if maximal valorization of the olfactory-gustative potential resulting from the release and conversion of volatile thiols is required (Curtin et al., 2008) (Fig. 4.4).

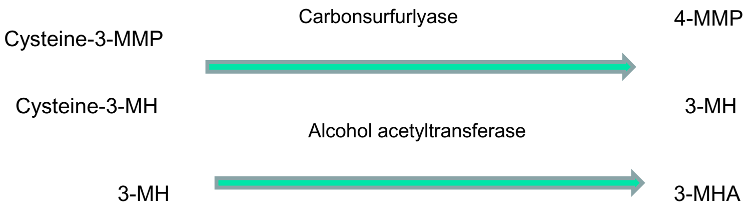

Fig. 4.4 Synthesis of volatile thiols by enzymatic hydrolysis of cysteinylated precursors under the action of selective yeast cells (Curtin et al., 2008).

The compounds 4MMP, 4MMPOH, and 3MH are released into wine from their grape-derived cysteinylated precursors during AF (Darriet et al., 1995; Tominaga et al., 1998a,b; Peyrot des Gachons et al., 2000, 2005). A major portion of the precursors remain nonhydrolyzed, and that the free 3MH is already present in the decantation, perhaps due to the action of the indigenous flora on these musts (Agosin, 2013). The rate of transformation of bound thiol precursors initially present in the grape into free thiols in the actual wine is very low, generally less than 5%–10% (Agosin, 2013). The β-lyase enzyme was established to be involved in the release of 4MMP as well as 3MH (Villas-Boas et al., 2014). The co-fermentation with *P. kluyveri* increases the varietal thiol concentrations in wine (Anfang et al., 2008; Zott et al., 2011) and the effect of multiple yeasts co-inoculations offer a more complex sensorial profile (King et al., 2010).

Diammonium phosphate (DAP) as nutrient is not recommended because it generates a reduction in the production of volatile thiols even if this inorganic compound is associated with other compounds in more simple or more complex nutritional formulas (Salmon, 2010).

Only the ingestion of glutathione and directly assimilable amino acids present an impact on the yeast cells multiplication and limits the adverse effects caused by the oxidation of proteins and amino acids inside the viable yeast cells (Ehsani, 2015).

The presence of O_2 induces the oxidation process of amino acids that causes an oxidative metabolism of yeast cells with negative influence on the odorant constituents of the fermentative medium that diminishes its concentration (Ehsani, 2015).

The intake of directly assimilable amino acids and glutathione has a favorable impact by facilitating cellular multiplication and limiting the adverse effects caused by the oxidation of proteins and amino acids inside the yeast cells in the presence of oxygen (AEB-Spindal, 2013).

This can be achieved by the use of nutrient addition obtained by yeast cell walls resulted from enzymatic and thermal autolysis of inactive yeast cells during AF, rich in glutathione, amino acids and enriched with B1 vitamin (AEB-Spindal, 2013).

The evolution of GSH during AF is still unclear. When compared to GSH levels initially present in grape juice, the levels in wine have been reported to be either lower (Du Toit et al., 2007; Patel et al., 2010; Coetzée et al., 2013) or higher (Park et al., 2000a,b; Andujar-Ortiz et al., 2012; Fracassetti, 2010).

AF temperature also has an influence on the release of thiol compounds. Recommended temperatures values are from 18 to 20°C which allow for a stronger enzymatic hydrolysis of cysteinylated precursors in free varietal thiols typical of Sauvignon blanc under the action of the appropriate enzyme equipment of selected yeast strains

(Masneuf-Pomerade et al., 2006). Howell et al. (2004) found that at 28°C some yeast strains released 100-fold more 4MMP than at 18°C. Although only small amounts (1.4%–4.2%) of aroma precursors are transformed during AF (Peyrot des Gachons et al., 2000; Howell et al., 2004), these concentrations of volatile thiols still contribute significantly to the overall sensory profile of the resultant wine.

4.5.4 The Particular Case of MLF for White Wine

General informations. Although global warming should reduce the interest in promoting MLF in white wine, on the contrary, an increased interest is seen in order to obtain dry white wines with the most complex and interesting sensory profiles. MLF represents the conversion of the malic acid of wine into lactic (Renouf, 2013). It is considered a secondary fermentation conducted by lactic acid bacteria (LAB), mainly *Oenococcus oeni*, in most red wines and some white and sparkling wines. MLF is a decarboxylation process where L-malic acid is converted to L-lactic acid with the production of CO_2 (Du Toit et al., 2013) and the citric acid is converted în diacetyle (fresh butter) (Croitoru, 2005). This process takes place in an increasing proportion of white and sparkling wines, due to the noticeable improvement in their microbiological stability and organoleptic characteristics (Bartowsky et al., 2015).

Influence factors. Many factors can influence the dynamic of MLF, such as: ethanol concentration (exceeding 15% v/v), low pH (less than 3.2), low temperature and SO_2 concentration (more than 50 mg/L), lysozymes, phenolic compounds, medium chain fatty acids, yeast-bacteria interactions, and nutrient availability (Lerm et al., 2010; Bartowsky and Borneman, 2011).

The production of flavor and aroma compounds is a result of the metabolism of grape constituents, such as sugars, amino acids and organic acids, and/or the modification of grape—and yeast-derived aroma compounds (Swiegers et al., 2005a; Bartowsky and Borneman, 2011). The amount of total esters produced after MLF differed according to the yeast strain used. The esters produced that differed significantly were ethyl lactate, ethyl acetate, ethyl caprylate, ethyl-3-hydroxy butanoate, ethyl phenyl acetate, and diethyl succinate (Du Toit et al., 2013).

The most common approach to MLF is the inoculation of selected LAB (Lasik, 2013). *O. oeni* is the best adapted lactic bacteria used for MLF. *Lactobacillus plantarum* has now included in MLF starter cultures, especially for high pH wines, and for co-inoculation with yeast (Du Toit et al., 2011; Lerm et al., 2010). It was shown in different studies that *L. plantarum* can, under specific conditions, perform MLF just as well as *O. oeni*. *L. plantarum* possess different genes and enzymes that can lead to different wine aroma profiles (Du Toit et al., 2011; Lerm et al., 2011). The biggest impact from using *L. plantarum* is related to the release of monoterpenes due to ß-glycosidase activity (Lerm et al., 2012).

White wine applications. Usually, MLF applies to some wines of the Chardonnay variety with a more complex sensory flavor that associates beside the fruit-floral character, a pleasant freshness (due to the reduction of excessive acidity specific to the variety, by transforming the malic acid—green character) into acid lactic acid velvety and softness and an interesting nuance of fresh butter (resulting from the metabolism of citric acid in diacetyl) (Croitoru, 2005, 2008a).

The influences of pH and ethanol. Knoll et al. (2011) investigated the influences of pH and ethanol on MLF and the volatile aroma profile in Riesling and Chardonnay wines using two different *O. Oeni* strains. And observed an increase in such fruity esters as ethyl acetate, ethyl propionate, and ethyl butyrate. Lower pH resulted in greater increases in total fruity esters. For monoterpenes, the content of trans- and cis-linalool oxide and α-terpineol increased with lower pH values, and the linalool content increased with higher pH (Du Toit et al., 2013).

Level of citric acid metabolized. Researches done on the level of citric acid metabolized at the end of MLF in northern dry white wines, in order to ensure the butter character preferred by a certain segment of consumers, are not many. The wines tested were Chardonnay, Sauvignon blanc, and two Romanian grape varieties (Crâmpoşie and Fetească albă). The used doses were 0.3, 0.5, and 0.7 g/L compared to the untreated control. All the tested wines were completely dry (sugars <2 g/L) in order to protect the citrate-lyase (which ensures the metabolism of citric acid) against the repressive action of glucose (Weinzorn, 1985). Citric acid was added in dry white wines after the premature racking; the preservation on the lees of the wines would have led to the decrease in the diacetyl content as a result of its reduction to acetoine and 2,3-butandiol. According to some authors (De Revel et al., 1996), the diacetyl content in the wine at the end of the AF would depend on the ability of the yeast strain used to reveal an enzymatic activity of diacetyl reductase and acetoin reductase. For all grape varieties, only the samples treated with 0.3 g/L citric acid were accepted by the tasters (Croitoru, 2005). The Chardonnay highlights a nice buttered character well associated with a floral note and persistence in the mouth with a slight decrease in fruitiness. The Sauvignon blanc has a buttered character that affects the olfactory typicity of the grape variety. For the Crâmpoşie grape variety, the buttered character diminishes the freshness characteristic. In the case of Fetească albă grape variety, the buttery character reinforces the finesse and the gustative complexity and decreases the typicity (Croitoru, 2008a).

Controlling MLF is an important issue to produce white wines with aging potential. Bacterial inoculation reduces the amount of time available for indigenous flora to multiply. Early MLF in a Chardonnay prevents the possibility of an oily wine (Gerbaux, 2016). Bacterial inoculation is a way of preventing biogenic amines from appearing in finished wines (Gerbaux, 2016). Jackowetz and Mira de Orduña (2012) suggested that microbiological wine stabilization 1 week after malic acid depletion is an effective strategy for maximum removal of SO_2 binders while reducing the risk of possible post-ML (malolactic) spoilage by *O. oeni* leading to the production acetic acid and biogenic amines.

Inoculation techniques for MLF traditionally occur after the completion of AF (in sequential inoculation) using commercial starter cultures; the inoculation with LAB and yeast at the beginning of AF (co-inoculation/simultaneous inoculation) is now an alternative for especially high pH and high proalcohol wines (Hansen, 2014, 2015). Knoll et al. (2012) evaluated four different MLF inoculations, from co-inoculation, 40% of AF, 60% of AF to sequential, using two different *O. oeni* starter cultures. The strain differences were more profound in co-inoculation and 40% of AF where there was a clear separation between the two strains and the esters they produced (Du Toit et al., 2013).

Sequential inoculation may be delayed or late. In the case of delayed sequential inoculation, viable live yeast cells and LAB are no longer living together; in the case of late inoculation, there may be a gap between viable cells of selected yeast and LAB for up to several months (from autumn to spring). Bacterial inoculation can be performed at various stages related to the AF stage (Hansen, 2014, 2015).

Reverse inoculation consists in inoculation with LAB prior to start the AF by inoculation with selected yeast (Hansen, 2014, 2015).

Co-inoculation consists of inoculating with LAB immediately after AF starting with inoculation with the selected yeast suspension or shortly after administration of this suspension. This co-inoculation may be early or late when the gap between the two biological fermenting agents is higher (Knoll et al., 2012; Guzzon et al., 2013; Munoz et al., 2014).

Early inoculation. LAB administration is applied in the last third part of the AF, when the density of the must is low to approximately 1020 g/L. It is sometimes confused with early co-inoculation. Rosi et al. (2003) showed an immediate and extreme decrease in LAB cell counts when the wine was inoculated midway through AF, decreasing as low as 10^4 CFU/mL in the first 6–8 days after inoculation then increasing again to 10^6 CFU/mL, at which point malic acid degradation began (Du Toit et al., 2013).

Sequential inoculation. LAB administration is done in the final stage of AF, immediately after separation of marc. Some of the literature suggests that sequential inoculation could be a means to avoid such problems as antagonistic yeast-bacteria interactions potentially associated with simultaneous inoculation (Lerm et al., 2010). Due to the completion of AF, the lower residual sugar concentrations that reduce the risk of acetic acid production are another advantage of sequential inoculation (Costello, 2005).

Late inoculation. LAB administration takes place after finishing the AF in wine but only after complete metabolism of sugars. This late inoculation involves a long-lasting shift between the two biological fermenting agents (Hansen, 2014, 2015).

Immobilized LAB. The *O. oeni* may be immobilized on different natural materials (corn cobs, grape skins, and grape stems) and used to induce MLF in white wines. Free biomass is higher than in the fermentations containing only free cells, demonstrating that the support contributes for a better performance of the bacteria (Genisheva et al., 2011).

4.6 The Postfermentation Phase of Dry White Wines

Dry white wine is that wine which has completed the fermenting processes, without always having a definitive sensory profile. This technological stage aims to provide antioxidant protection in order to preserve the odorant profile, improve the taste properties of some efficient wines and the biological stabilization of the resulting wine.

4.6.1 Specific Technological Elements to the Postfermentation Phase of White Winemaking

Antioxidant protection of the new wine. It is necessary to protect the wine with inert gases (CO_2 or nitrogen), especially when the tank is subjected to thermal variations affecting free varieties (high potency oxidation and high volatility compounds). Low doses of SO_2 or ascorbic acid treatment are not recommended because it favors the formation of compounds with high oxidation potential, of which the sotolone (curry odor) is the most representative.

Pinking phenomenon of white wines. Pinking is used for the salmon-red blush color that may appear in white wines produced exclusively from white grape varieties. The origin of the pinking phenomenon in white wines is the presence of anthocyanins (mainly malvidin-3-O-glucoside). The appearance of pinking in white wines after bottling is due to the lowering of free sulfur dioxide, which leads to an increase of the relative amount of the anthocyanins red flavylium form and their polymerization, resulting in the formation of colored compounds resistant to pH changes and sulfur dioxide bleaching (Silva et al., 2014).

The phenolics compounds. The presence of flavan-3-ols such as (+)-catechin and (−)-epicatechin accelerates the decrease in volatile thiol concentrations even at low concentrations, because of their combination with glutathione which cannot provide them antioxidant protection; in this context, the concentration in volatile thiols can be reduced gradually due to the progressive decreasing of glutathione; this undesirable phenomenon can also affect dry white wines rich in volatile monoterpenols (Nikolantonaki et al., 2010).

A total of 26 phenolics including hydroxycinnamate derivatives and minor compounds resulting from oxidation processes of a white wine without adding sulfites, after 12-month storage were reported by Pati et al. (2014).

The glutathione content. Recent research has demonstrated the importance of its presence in wine in ensuring a durable and effective protection of varietal free-range flavors typical of the variety. During wine aging, the presence of GSH has a protective effect on various impact aroma compounds, including volatile thiols (Lavigne-Cruege and Dubourdieu, 2002; Dubourdieu and Lavigne, 2004; Ugliano et al., 2011), esters, and terpenes (Papadopoulou and Roussis, 2001, 2008; Roussis et al., 2009). Fracassetti et al. (2013) investigated the interaction between sulfur dioxide, glutathione (GSH), and certain phenols in the presence of oxygen in a synthetic wine and in clarified Sauvignon blanc wine and found an increase in glutathionyl caffeic acid, as well as in oxidized glutathione (GSSG); the oxygen consumption rate was influenced by the SO_2 content, as higher SO_2 concentrations led to significantly higher consumption rates in both the synthetic wine and the clarified wine. The GSH-IDY (Optimum White) addition, made within the first third of AF, could lead to elevated wine GSH levels, provided the supplementation is done early during AF (Kritzinger et al., 2013).

Other treatments. By using a correction of acidity (with tartaric acid up to 0.5 g/L or citric acid up to 0.3 g/L) and treatment with high purity microgranulated arabic gum at doses of 40–80 g/hL, depending on the tasting evaluation reported (AEB-Spindal, 2013).

In order to preserve the volatile profile of white wines, a free SO_2 content of 35–40 mg/L is necessary (Ribereau-Gayon et al., 2004).

Results found by Coelho et al. (2015) indicated that SO_2 added to the must subsequently influenced white wine chemistry into three distinct sulfur dioxide dose-dependent aging mechanisms.

4.6.2 Antioxidant Protection Treatments of Volatile Monoterpenols and Valorization of Their Precursors

The enzymatic treatment needed the release of the odorant monoterpenols from the precursors extracted from the berries skins during the prefermentative stage. It is effective only if the first enzymatic treatment has been effective and substrate for the second enzymatic treatment. It is recommended to be applied when the glucose content has decreased below 20 g/hL (glucose presents an inhibitory effect on enzymatic activity) and the temperature is still above 15°C. The recommended dose is 5 g/hL and the duration is at least 2–3 weeks. The final sulfitation is recommended only after the treatment is complete. The protection of the quality and intensity of the varietal odorant character is achieved by using degraded autolyzed cell wall derivatives (with β-glucosidase activity) used at doses of 40–80 g/hL (Ehsani and Moine, 2015).

It has been proven that the use of DYP preparations (with or without GSH) reduces the loss of certain terpenes during the accelerated aging of model wines. DYP preparations contained small peptides (tripeptides) with methionine, tryptophan, and tyrosine, which seem to be effective in the preservation of some terpenes during model wine aging. Because of the instability of GSH in wines (easily oxidized, fast combination with polyphenols, etc.), this finding could be of technological interest, assuming the higher stability of these antioxidant peptides when used, for example, after wine bottling (Rodríguez-Bencomo et al. (2014).

4.6.3 Antioxidant Protection Treatments of Volatile Thiolsand Valorization of Their Precursors

Data from literature recommend maturation on the initial yeast sediment which gradually generates new amounts of glutathione in a strongly reducing environment for the preservation of the aromatic character of Sauvignon blanc wines, antioxidant protection could be more effective by using an exogenous addition of yeast derivatives rich in glutathione and peptides at a dose of 30–40 g/hL (Kritzinger et al., 2013).

4.7 Wine Aging Stage

Several ways can be applied to dry white wines for aging. In the case of white wines, the influence of the aging process applied on its sensory profile does not affect its fruity and olfacto—taste typicity.

4.7.1 Traditional Aging Processes

It refers to the aging of dry white wines in oak barrels and reveals notable differences between dry white wines varieties (Jordão et al., 2005) found that the temperature used during wine aging (cca. 12°C) appears to have less influence on ellagic tannins degradation, probably due to the regulation of oxidative reactions. Vescalagin and castalagin were the most abundant individual ellagic tannin compounds measured, followed by grandinin and roburin E, while roburin D was the ellagic tannin least detected in all extraction processes.

The case of Chardonnay dry wines. Depending on the desired sensory profile, maturation can be done in US oak barriques (the wine extracts the predominant flavor of coconut and vanilla) or French European barriques (the wine extracts mainly elagotanins). A successful Chardonnay should be consisted of 1/3 fruitful—floral (no oak), 1/3 wine with fresh butter aroma (which made MLF in barriques or in stainless steel tanks), and only 1/3 of the wine aged with oak wood (Croitoru, 2008b).

Case of Sauvignon blanc dry wines. Expert advice is contradictory: most exclude the use of new oak barrels because they allow too intense natural oxygen transfer to the environment), but exclude the total elimination of the primary autolyzed yeasts sediment because its disappearance means the elimination of a natural progressive source of glutathione production that is excellent protector for free varietal thiols typical for Sauvignon blanc wines. Coetzée and Du Toit (2015) reported that various interactions occur between both volatile and nonvolatile Sauvignon blanc wine constituents, and these interactions can change as the concentrations change.

Case of other grape varieties rich in volatile monoterpenols. In these cases, the maturation time may be only 4–8 weeks, to avoid the intensity of the oak character (rich in spices) to eclipse the fruity, freshness, and aromatic character of the matured wine (Croitoru, 2008b; Bompas-Denis, 2015).

Cryomacerated Minutolo grapes were used under reductive conditions and the resulting wine was aged for 12 months in glass container and in three types of amphorae (raw, glazed, and engobe). According to the principal component analysis (PCA), the wines were grouped as a function of the type of container where were aged (Baiano et al. (2015).

4.7.2 Combined Aging Processes

In this group included the maturation of the yeast first sediment and enzymatic maturation on the second yeast sediment collected from the surface of the first sediment (Croitoru, 2008b). The release of mannoproteins and the reduction of the polysaccharides with high molecular weight are most wanted processes. From sensorial point of view, an accentuation of smoothness and volume in the oral cavity should be observed.

Maturation on yeast sediment. Yeasts can produce more mannoproteins during and after AF, and these compounds can be released either during fermentation or through enzymatic action during autolysis when the wines are left on the sediment (Giovani and Rosi, 2007; Rodriguez-Nogales et al., 2012).

Enzymatic maturation. β-Glucanases can hydrolyze the cell walls and release mannoproteins and other carbohydrates during yeast autolysis, and storage of wines in contact with sediment can enhance the content of mannoproteins as these oenological yeasts may produce extracellular hydrolytic enzymes (Ferrer et al., 1998; Strauss et al., 2001; Moriwaki et al., 2014).

4.7.3 Innovative Wine Aging Processes

They are based on treatments with biotechnological preparation derived from autolyzed yeast cell walls which are thermoenzymatic degraded. The recommended dose is 40–80 g/hL and lasts for 3–4 weeks. It can be applied to any kind of dry white wine, depending on the desired sensory profile to be obtained (Croitoru, 2008b; Ehsani and Moine, 2015).

4.7.4 Unconventional Wine Maturation Processes

Accelerated aging vs conventional storage. The effects of storage time and temperature (accelerated aging) on Chardonnay white wines have been evaluated (Cejudo-Bastante et al., 2013). The authors reposted that both time and temperature factors (storage and accelerated aging) provoked common changes on the volatile profile of Chardonnay white wines.

The effects of aging and type of tank. Baiano et al. (2014) evaluated the effects of aging and type of container on physicochemical indices and on antioxidant compounds of "Falanghina" wines stored for 12 months in raw, glazed, and engobe amphorae, and in stainless steel tanks. During the whole aging time, the decrease of the antioxidant capacity ranged from 28% (raw amphorae) to 43% (stainless steel tanks).

4.8 Clarification and Stabilization of Aged Wines

Although they are approached together, they have been dissociated to reveal the novelty items from the literature for both technological stages.

4.8.1 Clarification of Aged Wines

Finning agents act on phenolic compounds in dry white wines by eliminating its phenolic compounds and to reduce the risk of oxidation and browning under the action of the formed quinones, which affect the volatile thiols (Nikolantonaki et al., 2010; Sarrazin et al., 2010). Bentonite is used in oenology to improve the limpidity and the stability of wine and to predict the formation of deposits in the bottle. Bentonite removes protein according to the matrix parameters, but others have described it as selective for some protein classes. For the Chardonnay and Sauvignon blanc tested in the study, fining with specific bentonite labels to remove select residual proteins, some of which are potential allergens, was very promising.

The removal of a single protein by bentonite affected the wine's thermal stability (Lambri et al., 2012).

The effect of bentonite treatments on the aroma compounds in white wine depended on the chemical nature and initial concentration of the volatile compounds and on the abundance and nature of proteins in the wine. In general, when low bentonite concentrations (20 g/hL) are applied, the concentrations of most aromatic compounds are not significantly affected (Lambri et al., 2010).

The use of milk- and egg-derived fining agents for wine fining can cause allergic reactions and must be included on wine bottle labels.

Cosme et al. (2012) suggested that pea protein could be an alternative for potassium caseinate, but it is less effective at reducing the white wine browning potential.

In recent years, for dry white musts and wines clearing, finning agents extracted from wheat, potato, and peas were evaluated (Renouf et al., 2013).

Protein extracts from autolyzed yeast (able to reduce the turbidity of white wines), but also chitosan and chitosan-glucan extracted from *Aspergillus niger* which, in addition to clearing, can remove some metals (Al, Pb, Fe, Cd, etc.) and mycotoxins (ochratoxin A), according to the recent research (Bornet et al., 2007; Bornet and Teissedre, 2008).

During the settling and clarifying of white wine must a decrease in MP concentration was notified (Roujou de Boubée et al., 2002).

4.8.2 Stabilization of Aged Wines

One of the major defects in bottled white wines is the formation of haze or deposits (aggregation of proteins) that can appear during transport or storage. Besides the temperature, the two other physicochemical factors that affect the stability of wine proteins are the pH and the ionic strength (Dufrechou et al., 2012, 2013; Marangon et al., 2011).

The impact of wine polysaccharides on wine protein aggregation at room temperature was studied. Polysaccharides modulated the aggregation kinetics and final haziness, indicating that they interfere with the aggregation process, but could not prevent it (Dufrechou et al., 2015).

The compounds released during fermentation as well as those from yeast cell walls were found to be highly dependent upon each yeast strain used during AF. The addition of β-glucanase preparations could be important to improve beneficial effects of the released components from yeasts (Giese et al., 2016).

Several studies have investigated the effectiveness of several proteolytic enzymes in free form as a treatment alternative to bentonite fining for wine stabilization. Benucci et al. (2014) who tested the feasibility of stem bromelain, free or immobilized on a chitosan support, to reduce white wine protein haze potential, found that immobilized bromelain appears a suitable treatment alternative to bentonite fining for white wine haze stabilization. Ascorbic acid can be used as a supplement to sulfur dioxide in winemaking in order to preserve sensory and analytical parameters of white wines (Smrcka and Baron, 2016).

Fruity white table wines are sensitive beverages whose flavor stability during storage and aging is a major concern in the wine industry (Ferreira Lima et al., 2016). Schneider (2016) reported that after fermentation, the time of filtration, careful protection against uncontrolled oxygen uptake before, during and after bottling, the rational use of sulfur dioxide and ascorbic acid, the choice of the bottle closure, and the storage temperature are the most important technical variables affecting aging reactions.

4.9 Conclusions and Final Recommendations

By applying innovative biotechnology for the production of dry white wines presented in this chapter requires the separation of the two grape varieties used as raw material in predominant monoterpenic (Reynolds and Balint, 2014; Gambetta et al., 2014) and predominant thiolic varieties (Lund et al., 2009; Benkwitz et al., 2012a,b; Coetzée and Du Toit, 2012) although by their agrobiological nature these varieties reveal that a quite strict delimitation cannot be established. The approach presented, in our opinion, is the first in the literature. We leave the specialist reader the pleasure of constructive, reasoned, and objective critical analysis of the subject.

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5

DEVELOPMENT OF RED AND WHITE WINES FROM LOCALLY ADAPTED GRAPE CULTIVARS USING INDIGENOUS YEAST

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5.1 Introduction

India is the world's second largest producer of fruits with 57.73 million tons after China with its anticipated estimate touching 98 MT by 2020–21. Currently, grape crop is grown over an area of 118,000 ha with an annual production of 2.4 million tons (NHB, 2015). The fermented product of grapes, that is, wine has probably been an important way of consuming grapes (McGovern et al., 1995). The four biggest wine makers, arranged by world positioning, are Italy, France, Spain, and United States (OIV Statistics, 2015). In India, verifiably wine has been made for 5000 years. Early European voyagers introduced both red and white wines to the Mughal courts in the 16th and 17th century. In the 19th century, vineyards were established in Kashmir and at Baramati in Maharashtra and various Indian wines were served to the guests during Great Calcutta Exhibition of 1884 under British influence. Nonetheless, Indian vineyards were completely pulverized by obscure reasons in the 1890s. Since then Indian wine industry is in its infancy and is restricted primarily to Maharashtra, Karnataka, and Goa to some extent but grew by 25%–30% between 2009 and 2012 (Patil, 2008; Kocher and Nikhanj, 2011). The main constraint in the growth of wine industry is, however, lack of suitable grape varieties and to some extent dependence on imported wine-producing yeasts. The selection of suitable grape varieties and their optimum maturity stage having the potential to make wines of high quality is a continuous work in all the wine-producing countries. This is of particular concern in India where wine-producing varieties are lacking. Only wineries like Champagne Indage (CI), Grover Vineyards, and Sula

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Vineyards are producing quality wines from varieties of grapes such as Sauvignon, Merlot, Pinot, Shiraz, Zinfandel, etc. (Karibasappa and Adusle, 2008) that are all exotic varieties and have been adapted to grow under Indian conditions. As far as Punjab is concerned, in spite of 61,618 ha under fruits cultivation with 1,055,408 MT annual production, no winery has been set up yet. The reason behind it seems to be the monoculture of Perlette which is a table variety. Now the varieties like Punjab MACS purple, Chardonnay, Merlot, Shiraz, Chason, etc. have been adapted to grow under Punjab conditions. Therefore, evaluation of such grape varieties for wine production and optimization of wine fermentation process technology is pertinent to establish a winery in north India region.

5.2 Wine, a Nutritional Drink

Wine, an ethanolic beverage, is produced through ethanolic fermentation of fruit juices by *Saccharomyces cerevisiae*, a traditional fermenting yeast that metabolically produces carbon dioxide as well (Combina et al., 2005). The nutritional role of wine is important since its average contribution to total energy intake is estimated to be 10%–20% in adult males (Macrae et al., 1993). Wine provides various antioxidants in the form of phenolic compounds, aromatic compounds, flavonoids, non-flavonoids, anthocyanins, ascorbic acid, tannins, stilbenes, resveratrol, and many other bioactive compounds. Two most significant constituents of wine are: water (75%–90%) and ethyl alcohol (8%–14%, v/v or more). However, variation in the composition of wine depends on the substrate used, processing method, and fermentation conditions. Alcohol content, flavors, aromas, and bouquet are the chief characteristics used to describe a particular wine. People's attention to the beneficial effects of moderate wine consumption was started in 1990s when "French paradox" reported "Although, the French diet is richer in saturated lipids compared to other countries diets, the level of mortality is less due to coronary heart disease because of daily wine consumption" (Renaud and De Lorgeril, 1992). Alcohol itself in moderate amounts acts as a stimulant to maintain blood flow in the body while wine polyphenols and other bioactive compounds contain antioxidant properties (Bors and Michel, 2002). Epidemiologically, it is shown that wine consumption may ensure against the frequency of numerous ailments of present-day society—cardiovascular sickness, dietary malignancies, diabetes, hypertension, macular degeneration and maintenance of bone thickness. The advantages of cancer prevention agents are more articulated in red wines as the levels of phenolics and anthocyanins are higher than that in white wines as previous are subjected to coming about because of skin maceration treatment before aging. In short, a glass of wine is equal to a glass of health, containing plenty of beneficial bioactive compounds.

5.3 Grapes

Grape (*Vitis* sp.) is a commercially important fruit crop of India. It is a temperate crop belonging to family Vitaceae and has got adapted to subtropical climate of India. According to the Food and Agriculture Organization (FAO), 75,866 km^2 of the world are dedicated to grapes. In India, an annual production of 2.4 million tons of grapes was reported during the period 2015–16 with Maharashtra, Karnataka, Tamil Nadu, Punjab, Andhra Pradesh, Madhya Pradesh as major grapes-producing states. The area dedicated to vineyards is increasing by about 2% per year (Kocher and Nikhanj, 2016).

5.3.1 Grape Berry Composition

Fresh grape juice consists of 70%–80% water and different dissolved solids. These soluble solids include plentiful organic and inorganic compounds. Each component of the grape berry contains different classes of phenolic compounds, contributing to the sensory properties of the wine. Proanthocyanidins and monomeric flavonols are found mostly in the grape skins and seeds that contribute to the bitterness and astringency of the wine. Anthocyanins are red pigments and the primary source of pigmentation in red wine (Harris et al., 1968). From winemaking point of view, the important group of compounds includes the following: total soluble solids, organic acids, phenolic compounds, nitrogenous compounds, aroma and flavor compounds, and minerals.

Sugar buildup in grape berries is an imperative phenomenon which determines the amount of alcohol in the final wine. In addition, total sugar content is a significant fruit quality factor in table grapes cultivars. Glucose and fructose are the predominant sugars in grapes with only traces of sucrose. The accretion of sugars (glucose and fructose) within the cellular medium of berries, specifically in the vacuoles, is the characteristic feature of the grape berries' ripening process. It is a major profitable consideration for the grape grower, winemaker, and dried grape producer. Sugar quantity is an indicator used to evaluate ripeness and to harvest the grape berries. In addition, as mostly the sugars are fermented into alcohol during the winemaking, the quantity of sugar, known as "must weight," assess the content of alcohol in the wine.

Tartaric and malic acids are the principal organic acids of the grape berry, contributing to approximately 90% of the total berry fruit acidity. Both acids are produced in the berry, along with small amounts of citric acid and other non-nitrogenous organic acids. They are responsible for the tart taste and have a marked influence on wine stability, color, and pH. Grape berries vary in the relative amounts of these acids depending on cultivar, climatic conditions, and cultural practices (Navarro et al., 2001; Liu et al., 2006).

Phenolics are important for the development of a quality wine, since it improves aromatic as well as nutritive profile of the resulting wine. In particular, anthocyanins are considered most important phenolics for color in red wines and tannins in both red and white wines. Berry skin and seeds contains tannins and other pigments and the pulp contains only juice. Phenolic compounds in the berries increase during the ripening of the berries and their amount varies depending on the grape cultivars and agroclimatic conditions. In biological perspective, epidermis' insoluble cutin and lignin of seed coat are phenolics, which are essential as skin tannins, pigments, and seed tannins. However, grape berry's soluble phenolics are important in winemaking (Kocher and Nikhanj, 2016).

Aroma and flavor compounds provide the wine its unique taste. Both contribute to the bouquet, overall mouthfeel, and quality of the wine. Grape berries and wine are known to have various aromatic and flavor compounds with a diversity of mechanisms involved in their development. Grape berry metabolism, biochemical reactions occurring prior to fermentation, fermentation metabolism, and chemical and enzymatic reactions occurring after fermentation adds flavor to the resulting wine. During aging process of the wine in vat, barrel, and bottle additional flavor compounds are added from the oak corks and barrels. Volatile metabolites consist of esters, volatile fatty acids, higher alcohols, carbonyls, and sulfur compounds, giving wine its vinous character. Many odoriferous compounds are released into oak barrel-aged wine during the storage process. Odoriferous compounds released from grapes play an extra decisive role in the quality and regional character of wines.

5.3.2 Selection of Grape Berries

The most dependable criterion to harvest grape berries for wine production is the estimation of biochemical parameters. From a biochemical point of view, the maturity stage is determined by certain quality indicators such as sugar accumulation, acid content, sugar to acid ratio, and phenolic compounds that contribute to the quality of the produce. Achievement of the optimum concentrations of these components is governed by viticulture strategies that can be applied to optimize ripening (Coombe and McCarthy, 2000; Carmona et al., 2008). An understanding of the critical accumulation stages of such components during berry maturation is needed to regulate grape rising practices and thus modify wine typology (Conde et al., 2007). Grapes having optimal composition for producing a particular type of wine are known to be technologically mature (Antoce, 2007). Technological maturity refers to accumulation of large amounts of sugar and reduced excessive acidity in order to achieve a balanced ratio between the two parameters. Glucoacidimetric index values (ratio between total sugar content and acidity) increase during maturation and at the ripening of the grapes, and for wine grape varieties the desirable index has values between 27 and 34 (Antoce, 2007). Brix-pH index ($\text{Brix} \times \text{pH}^2$) of the berries (at the time of harvest) should be in the range 200–270 for wine production. Adequate levels of phenolics, aromatic, and flavor compounds along with sugar and acid levels are also estimated during harvesting of grapes to improve the visual, aromatic, and overall quality of the resulting wine.

Grape berries (10 clusters from each vine and 10 berries from each cluster) cv. Punjab MACS purple and H-144 from vineyard were hand harvested and randomly collected from the start of veraison to the fully matured stage at regular intervals of 3 days (in sterile plastic bags) and transported to the Industrial Microbiology laboratory, PAU, Ludhiana for further physicochemical analysis. The physicochemical analysis of grape berries performed included the estimation of total soluble solids (digital refractometer, ATAGO), total sugars (Dubois et al., 1956), reducing sugars (Miller, 1959), pH (Hanna HI96107), titratable acidity (Amerine and Roessler, 1983), ascorbic acid (AOVC, 1996), anthocyanins (Zoecklein, 2005), glucose, and fructose contents (HPLC), malic acid (Goodban and Stark, 1957), tartaric acid (AOVC, 1996), and total phenols (Malik and Singh, 1991).

Harvested grape berries (cv. Punjab MACS purple and H-144) were analyzed for various physicochemical parameters for the entire veraison period that continued for 19–20 days in the case of Punjab MACS purple and 12–15 days in H-144. Throughout the veraison period, TSS contents of both Punjab MACS purple (3.4–18.0°B) and H-144 (2.1–16.2°B) cultivars were found to be increased indicating improvement in the sweetness of the berries. Comprehensive analysis of sugar components showed that total sugars and reducing sugars were increased with increase in the TSS content from onset of veraison to fully matured stage. Glucose and fructose contents in the berries increased during veraison to fully mature stage ranging from 11.3 to 90.2 g/L in Punjab MACS purple and from 5.2 to 77.8 g/L in H-144, respectively. Results have shown that glucose predominates over fructose in early stages while fructose content was higher than glucose content in later maturity stages in both the cultivars (Thoukis and Amerine, 1958). At fully ripened stage G/F ratios were found to be 0.91 and 0.96 in Punjab

MACS purple and H-144 cultivars, respectively, that met the desirable range (Bisson, 2001) for wine production.

Due to hot weather and pre-monsoon rainfall under North Indian climatic conditions, grape cultivars showed a low °Brix content which maximally reached to 18°B in Punjab MACS purple and 16.2°B in H-144. However, processing of grape into wine requires an initial brix of 20–22°B so as to achieve a final ethanol content of 12%–13% (v/v). Hence, °Brix level needs to be accustomed to 20–22°B (by chaptalization) so that the desired ethanol levels may be obtained in the resulting wine. This is also imperative when there is climate change whereby grape cultivars suffer from pre-monsoon rainfall damage that led to a sharp decrease in the TSS (°B) levels as well as physical damage to berries, making them inappropriate for wine production. Recording of °Brix, pH, and acidity is an easy criterion for selecting optimum grape berries for wine production that includes the calculation of °Brix-acid ratio and °B-pH index, which should be in the range of 27–34 and 200–270, respectively (Bisson, 2001). During the veraison period, the Punjab MACS purple cultivar showed the Brix-acid ratio of 30 and Brix-pH index of 259, which were found in the desirable range for both the cultivars. H-144 cultivar showed the Brix-acid ratio of 32 and Brix-pH index of 259, which were also in the desirable range for wine production (Nikhanj et al., 2016) (Table 5.1).

Table 5.1 Physiochemical Characteristics of Grape Juice (Punjab MACS Purple and H-144)

Parameters	Punjab MACS Purple	H-144
TSS	18.0 ± 0.7	16.2 ± 0.1
Titrable acidity (% w/v)	0.6 ± 0.2	0.5 ± 0.2
pH	3.8	4.0
Brix-acid ratio (27–34)[a]	30	32.4
Brix-pH index (200–270)[b]	259.9	259.2
Tartaric acid (g/L)	4.1 ± 0.2	4.5 ± 0.1
Malic acid (g/L)	3.2 ± 0.4	3.6 ± 0.6
Total phenols (mg/100 mL)	162 ± 1.9	120.4 ± 2.87
Anthocyanins (mg/100 mL)	23.6 ± 0.3	0.68 ± 0.14
Ascorbic acid (mg/100 mL)	1469 ± 3.1	1284 ± 3.2

All values are mean of triplicates ± standard deviation.

[a] Desirability level; index-$(pH)^2$ X Brix.

[b] X Brix; ratio-Brix/Acidity.

5.4 Prefermentation

Once grapes are harvested, phenolic profile of the wine depends on processing protocols followed in the winery. Prior to fermentation, various prefermentative treatments like cold maceration, skin contact, pressing, and heat treatment of grape juice have been followed to enhance the color, aromatic profile, and nutrient contents of the mature wine. With the exception of pulp-derived hydroxycinnamic acids, phenolics derived from the skin and seeds make up the vast majority of the phenolic pool present in the wine. As anthocyanins are localized in the skin of grape cultivars, fermentation and maceration have a reflective effect on the amount of anthocyanins present in the final wine. Skin maceration generally prompts increased concentration of aroma compounds in the final wine, while the end result is influenced by maceration conditions as well as the fruit cultivar used (Selli et al., 2006; Sanchez-Palomo et al., 2007). Phenolic compounds from grapes and wines have also received increasing interest because of diverse health benefits attributed to them (Moreno et al., 2007; Katalinic et al., 2010; Parker et al., 2007). In addition, skin contact leads to higher color intensity and sensitivity to oxidation of white wines (Mattivi et al., 2002). Research has shown that in any maceration process, contact time and temperature are the two critical variables in determining the ultimate amount of anthocyanin present in the wine. Potassium meta-bisulfite is normally added to the juice for inhibiting the growth of unwanted microbes and extraction of anthocyanins by providing SO_2 that act as a solvent for anthocyanins. However, potassium meta-bisulfite must be added in small quantity, since it is a bleaching agent and it leads to discoloration. Phenolic compounds extracted during the maceration process improve the quality of wines by modifying their color, flavor stability, and aging behavior. They also contribute toward the sensory properties of wine, affecting color, astringency, and bitterness (Esti and Tamborra, 2006). However, skin contact increases the phenolic compounds of wines and in some cases may cause more astringent and bitter taste (Cabaroglu et al., 1997). For this reason, maceration conditions must be carefully chosen and standardization of contact time, temperature, and KMS concentration are necessary prefermentative parameters for effective extraction of phenolics, anthocyanins, and tannins into the grape juice.

In the present study, RSM was adopted in the experimental design as it emphasizes the modeling and analysis of the problem in which response of interest is influenced by several variables and the objective is to optimize this response. A five-level three-factor central composite rotatable design (CCRD) was employed. For each experiment, 300 mL of juice (taken in 500 mL capacity glass bottles) was subjected to different skin pretreatment conditions. The temperature of the treatment combinations was adjusted to the desired level (±0.5°C) for 16-h period (Kaur and Kocher, 2014). At the end of the pretreatment, the juice was centrifuged at 6000 rpm for 10 min and analyzed for its color ($OD_{600\ nm}$) w.r.t blank, anthocyanins, and total phenolics. Grape skin used for pretreatment was also analyzed for decrease in anthocyanins and phenolics contents.

Three responses viz. phenols, color, and anthocyanins were taken and all the models for three responses showed significant values of 135.01, 19.92, and 84.31 in Punjab MACS purple and 133.68, 181.45 and 265.20 in case of H-144, respectively (Nikhanj and Kocher, 2015). The adjusted R^2 values for all models are in reasonable agreement with their predicted R^2 values. The analysis of variance (ANOVA) of the optimization study indicated that the model term A (skin weight) was significant ($P < 0.05$) for all the responses for both the cultivars. However, the interactions between the skin weight and the KMS concentration (AC) for the phenols response and interactions between skin weight and temperature (AB) were found to be significant for the color and anthocyanins responses for the Punjab MACS purple cultivar. In contrast, no interaction was found to be significant in the pretreatment of H-144 cultivar juice.

5.5 Ethanolic Fermentation

Ethanolic fermentation is the principal fermentation, where yeasts convert sugars in grape juice (mainly glucose and fructose) into ethanol (the main metabolite of wine) and carbon dioxide. This process can be conducted either as an indigenous/wild fermentation, or as an induced/seeded fermentation. With indigenous fermentation, yeasts tenant of the grape juice initiate and complete the fermentation. With seeded fermentation, selected yeast strains, generally those of *S. cerevisiae* or *S. bayanus*, are inoculated into the juice at initial populations of 10^6–10^7 cells/mL (Fleet and Heard, 1993).

Most frequently fermentations are initiated by the growth of various species of non-*Saccharomyces* yeasts like *Candida, Debaryomyces, Hanseniaspora, Hansenula, Kloeckera, Metschnikowia, Pichia, Schizosaccharomyces, Torulaspora*, and *Zygosaccharomyces* depending on the type of grapes and their inherent microflora. Their growth is generally limited to the first 2 or 3 days of fermentation whereby ethanol concentration of (2%–5% v/v) is achieved at which the inherent microflora becomes ethanol sensitive and hence ineffective. Consequently, the strongly fermenting and more ethanol-tolerant species of *Saccharomyces* sp. inoculated in the must take over the fermentation.

S. cerevisiae used to ferment grape musts may earlier be subjected to a prefermentative skin treatment followed by a short aeration pulse to build the inoculum size to a desired level. It has been reported that the short aeration has no significant effect on the unsaturation index of the cellular fatty acids, although it increases the ergosterol/phospholipid ratio. This is reflected by an increase in the growth rate, viability, and fermentative capacity of the yeasts (Fleet and Heard, 1993). Stuck fermentation risks and development of various wine faults can also occur during this stage that can last from 5 to 14 days for primary fermentation. Fermentation may be performed in stainless steel tanks, which is frequent with many white wines like Chardonnay, in an open wooden vat, inside a wine barrel, and inside the wine bottle itself as in the production of many sparkling wines (Kunze, 2004; Robinson, 2006). Fermentation of the juice was traditionally conducted in large wooden barrels or concrete tanks, but most modern wineries now use sophisticated stainless steel tanks with facilities for process management. White wines are generally fermented at 10–18°C for 7–14 days or more, where the lower temperature and slower fermentation rate favor the retention of desirable volatile flavor compounds (Robinson, 2006). On the other side, red wines are fermented for about 7 days at 20–30°C, where higher temperature is necessary to extract color from the grape skins. In winemaking, temperature and pace of fermentation is an important concern with the levels of oxygen present in the must at the beginning of the fermentation. There are number of factors that affect yeast fermentation performance like yeast strain employed, fermentation temperature, media composition, pH, substrate concentration, mode of substrate feeding, osmotic pressure, ethanol concentration, membrane composition, etc. (D'Amore, 1992). Considering the impact of important fermentation parameters over the fermentation process, the present study was carried out to optimize postinoculation initial agitation rate, temperature, inoculum size, and nutrient supplementation.

In our study, statistical optimization of fermentation parameters [initial agitation rate (rpm), temperature (°C), inoculum size (%v/v), and diammonium hydrogen orthophosphate (DAHP) supplementation (mg/100 mL)] was carried out using RSM plan under design expert 9.0 software. Synthetic grape juice; namely Triple M media (Spiropoulos et al., 2000) having 22°B was taken in 250 mL capacity flasks (100 mL working volume) for each of the 30 combinations according to the RSM plan w.r.t inoculum size, DAHP supplementation, agitation rpm levels (24 h), and incubation temperature. Responses were studied in terms of ethanol production (%v/v), total sugars (%), and reducing sugars (%) at the end of fermentation process. Results have shown that maximum ethanol production (11.7% v/v) was achieved in the combination of 75 rpm initial agitation rate, inoculum size of 5% v/v, DAHP supplementation at 250 mg/100 mL, and fermentation temperature of 20°C. A model *F*-value of 857.77 implies that the model for ethanol response is significant. Among the factors, that is, A, B, C linear factors, AC, BC, BD, CD interactive factors and A^2, B^2, C^2, D^2 quadratic factors were found to be significant model terms. The "Lack of Fit *F*-value" of 4.38 implied that it is nonsignificant. The nonsignificant lack of fit of the tested model also indicated that the model was a good fit. The "Pred *R*-Squared" of 0.9934 was in reasonable agreement with the "Adj *R*-Squared" of 0.9976, that is, the difference is less than 0.2. By applying multiple regression analysis, the equation in terms of significant factors for the ethanol response is as follows:

Final equation in terms of significant coded factors for ethanol response:

$$\text{Ethanol} = +11.67 - 0.10^{*}A + 0.31^{*}B + 0.73^{*}C + 6.250E - 004^{*}AB - 0.17^{*}AC - 0.028^{*}AD + 0.46^{*}BC - 0.36^{*}BD - 0.20^{*}CD - 0.36^{*}A^{2} - 0.81^{*}B^{2} - 0.85^{*}C^{2} - 0.36^{*}D^{2}$$

Numerical optimization by RSM was done into two ways, keeping the fact that red wines are often fermented at higher temperature (approximately 25°C) while white wines are usually fermented at low temperature (approximately 20°C). In the first way, optimization was done for the red wine production keeping three parameters namely agitation rate, inoculum size, and temperature in range and DAHP at minimum concentration level for supplementation. In the second way, optimization was done for H-144 white wine production keeping agitation rate, inoculum size in range, DAHP supplementation and temperature at minimum level. Results indicated that synthetic media kept at initial agitation at 80 rpm for 24 h, having 6.5% (v/v) inoculum size supplemented with 150 mg/100 mL and fermented at 24.6°C led to 11.7% (v/v) ethanol production with the desirability of 97.4% for the red wine production from Punjab MACS purple cultivar. Numerical optimization for H-144 results showed that an initial agitation rate of 79 rpm, 6.0% (v/v) inoculum size, 150 mg/100 mL DAHP supplementation, and fermentation temperature of 21°C as optimum led to ethanol production of 11.5% (v/v) at the end of ethanolic fermentation with a desirability level of 91%.

Optimized results for red wine production were validated at the 5L scale of Punjab MACS purple grape juice that resulted in the 12.0% (v/v) ethanol production with 87% efficiency rate and final 0.02% total sugars level and no final reducing sugars indicating it a highly efficient ethanolic fermentation. The optimized results for white wine production were also validated at 5L scale H-144 grape juice that led to production of 11.2% (v/v) ethanol, 0.12% total sugars, and 0.035% reducing sugars at the end of fermentation (Nikhanj and Kocher, 2017).

5.6 Malolactic Fermentation

Malolactic fermentation (MLF) is a secondary fermentation process causing biological transformation of malic acid into lactic acid and CO_2, and the consequent reduction in acidity and increase in pH, accompanied by several aromatic and sensory changes in the wine. Secondary compounds production during MLF brings important changes in both the quality and composition of wine besides providing microbiological stability to the final wine. The MLF is nowadays considered as an essential part of fermentation especially in the case of red and some white winemaking. Increase in oenological interest in the MLF process and its implications in the improvement in quality of the wine make it a subject for new research studies focusing on the different parameters affecting the MLF development viz. inoculation stage, selection of bacterial culture, nutrient supplementation, etc. On the other hand, the main advantage and potential danger of the metabolism of lactic acid bacteria (LAB) is the possible synthesis of some compounds that could affect the food security, like the biogenic amines (Isabel et al., 2009; Araque et al., 2009).

Wine-relevant LAB responsible for the MLF comprise the LAB of genera *Pediococcus*, *Lactobacillus*, and *Oenococcus* with its only species, *Oenococcus oeni.* These bacteria, which equally occur as winery contaminants, are found in grape musts in low populations (10^3 cfu/mL), but generally most of them die during ethanolic fermentation. Only after completion of ethanolic fermentation, the bacterial population might increase and reach up to 10^6 cfu/mL when MLF occurs. As for yeast, the development of wine LAB during the vinification may be negatively affected by the low fermentation temperature, acidic pH, high SO_2, and ethanol concentration (Crespo et al., 2010).

The MLF leads to improvement in wine in terms of aroma and flavor giving smooth mouthfeel to the wine. The LAB influence aroma and flavor in the wine through the production of volatile secondary metabolites and modifications to grape- and yeast-derived metabolites (Davis et al., 1985). Laaboudi et al. (1995) observed difference between MLF and non-MLF wines and reported lower acidity level of MLF wines, though their flavor was not significantly modified. Certain LAB also degrades citric acid into various products of metabolism, among which acetoin compounds, diacetyl, acetoin, and 2, 3-butanediol are the important ones. A moderate diacetyl content is desirable in wine; however, excessive acetic acid, glucane, biogenic amines, and ethyl carbamate precursors are not desirable.

In the case of red wines, the concentrations of anthocyanins were found to fall during MLF (Garcia-Falcon et al., 2007). However, García-Falcón et al. (2007) observed the absence of correlation between changes in color density and monomeric anthocyanins content in two young Spanish red wines during a year of storage. Among other factors, the color of anthocyanins depends on its different components. According to Henick-Kling (1993), MLF increases the fruity and buttery aromas but reduces vegetable or grassy aromas. The formation and hydrolysis of esters during MLF may also lead to an increase in the fruity aroma and it is, probably, due to the action of LAB esterases responsible for the synthesis and degradation of these compounds. The reduction in vegetable or grassy aromas could be due to the catabolism of aldehydes by LAB (Osborne et al., 2000).

The MLF parameters viz. bacterial inoculation was optimized by inoculating bacterial culture at various stages (simultaneous, sequential, and postfermentative bacterial inoculation to ethanolic fermentation) of ethanolic fermentation while the inoculum size and supplementation of nutrients were optimized using RSM. Punjab MACS purple and H-144 juice had the malic acid content in the range 3.9–4.2 g/L which is known to give harsh flavor and tongue feel to the consumer. However, desirable level of malic acid in wine is 1–3 g/L. Therefore, to deacidify harsh flavor of malic acid to smoother flavor of lactic acid, MLF is done in the synthetic grape juice/grape juice using a standard culture *Leuconostoc oenos* NCIM 2219.

Inoculation stage (simultaneous, sequential, and postfermentative bacterial inoculation to ethanolic fermentation) was optimized by inoculating 5% v/v bacterial culture in 100 mL of synthetic triple M media (synthetic grape juice; with 4 g/L malic acid and 20°B, pH adjusted to 3.7–3.8 using KOH) in 250 mL conical flask and incubated at 22 ± 2°C for efficient MLF. Four different timings w.r.t. ethanolic fermentation (as explained in Materials and methods) were studied as follows:

- Control—No bacterial inoculation
- A1—Simultaneous inoculation with yeast culture (at 20°B)
- A2—Mid of ethanolic fermentation (at 10°B)
- A3—Just before the end of ethanolic fermentation (at 3–4°B)
- A4—After the end of ethanolic fermentation (at 0°B)

Results showed that bacterial inoculation after completion of ethanolic fermentation (A4) and just prior to completion of ethanolic fermentation (A3) had the highest degradation rates of malic acid as compared to the other three inoculation stages during same time period. Maximum degradation of malic acid from 4.0 to 1.31 g/L was reported by *L. oenos* at inoculation stage A4. A comparable degradation was also observed in the inoculation at A3 stage where *L. oenos* degraded malic acid to 1.37 g/L. However, ethanol production in A3 stage inoculations was lower than the A4 stage as bacterial culture was inoculated just before the completion of ethanolic fermentation (at 3–4°B), the resulting ethanol levels were 9.7% (v/v). Inoculation at A1 and A2 stages lead to low ethanol production as the bacterial cultures too consumed the sugars for their growth and other biochemical conversions thus making it unavailable to yeast for ethanol production. Low malic acid degradation rate as well as slow degradation process was observed in the A1 and A2 inoculation stages. pH and titratable acidity followed inverse relationship during the MLF. pH values increased from 3.8 to 4.0 while titratable acidity was found to decrease by an order of 0.1%–0.3% units (unpublished data).

After optimizing the inoculation stage, that is, after completion of ethanolic fermentation, optimization of bacterial inoculum size (%v/v) and amount of nutrient supplementation (yeast nitrogen base with amino acids) was statistically planned using RSM and executed at the scale of 100 mL using synthetic grape juice (having malic acid—4.0 g/L). After completion of ethanolic fermentation, the bacterial culture (*L. oenos*) was added at different rates according to the RSM plan with specified nutrient supplementation and incubated at 22 ± 2°C for MLF (Crespo et al., 2010).

The MLF was performed after the completion of ethanolic fermentation (at 2L scale for both cultivars keeping rest of wine as control for sensory comparison). Punjab MACS purple red wine (10.7% v/v ethanol; 4 g/L malic acid; 20.0 ppm total SO_2, and 8.0 ppm free SO_2) and H-144 white wine (10.6% v/v ethanol; 3.9 g/L malic acid; 25 ppm total SO_2, and 8.9 ppm free SO_2) were subjected to MLF under the optimized conditions (inoculum size—7.1% v/v and 243.2 mg/L YNB supplementation at 22°C) for 12 days that led to the reduction of malic acid to 1.65 g/L in Punjab MACS purple and 1.56 g/L in H-144 wine. Finally, prepared Punjab MACS purple red wine and H-144 white wine were kept for settling at 8°C for 24–36 h and then siphoned into glass containers with nitrogen sparging at 1.5 bar pressure for 10–15 s.

5.7 Postfermentative Analysis

Post primary and secondary fermentations, wines are subjected to aging in various types of containers, including stainless steel tanks and oak barrels, and decanted several times to remove sediments formed by sedimenting yeast and bacteria (lees). Further, additional steps may include addition of fining and stabilizing agents to remove compounds that can lead to color and aroma deviations or unsightly sediments or hazes (Ribéreau-Gayon et al., 2000). Among the findings, the removal of white wine proteins that can lead to hazes during storage by bentonite is important, as well as the softening of red wine phenolics by fining with proteins such as gelatin, casein, or isinglass (Ough, 1992). Wines may also be cold stabilized, that is, stored at cool temperatures for a certain time to favor the precipitation of unstable potassium tartrate crystals. While not imperative, many wines are also filtered before bottling in order to remove insoluble compounds and microorganisms that could lead to spoilage after bottling. Wine stability also relies heavily on the presence of the preservative sulfur dioxide (SO_2) which has antioxidant and antimicrobial activities.

5.7.1 Racking

Racking of wine is the process of sorting out wine from its sediment, or lees, and transferring the wine into another container using a siphon. Repeated racking produces the clarity required in wine, especially if it is aged in a barrel. The wine is repeatedly racked to leave behind less and less precipitate. During the repeated pouring, the wine is also given a chance to rid itself of the excess carbon dioxide produced from fermentation. Racking also helps in eliminating certain unpleasant reduction odors (H_2S), as well as iron and is also responsible for intensifying color of wine (Amerine and Roessler, 1983) and also contributes to long-term wine stability. Racking cones and racking siphons are usually used to rack the fresh wines. Small containers like carboys are usually racked by siphoning the wine off the lees with a piece of clear plastic tubing. Barrels and drums are often racked with a small pump and plastic tubing. Wineries use powerful electric transfer pumps and large diameter hoses made from food grade materials to rack their large stainless steel wine tanks. White wines are normally racked off the yeast lees shortly after the finish of alcoholic fermentation. Red wines are often left on their gross lees until ML fermentation is finished. Afterwards, they are racked for two to three times usually at 6-month intervals.

5.7.2 Nitrogen Sparging

Oxidation problems include enzymatic oxidation (causes browning of wine color), bacterial growth (causes spoilage), and pinking (affects white wine after fermentation). Permissible limit of oxygen in the wines is below 2 ppm. After fermentation, it is essential that wine be kept oxygen free to maintain its quality and prevent oxidation. Sparging removes dissolved oxygen and adjusts carbon dioxide levels of wine. Use of inert gases such as CO_2, N_2, and Ar has become very common in modern winemaking. The main purpose to use inert gas in the headspace (ullage) of a wine container is to protect the wine against oxidation and spoilage by yeast and bacteria. N_2 and CO_2 are the most commonly used inert gases in the wine industry today for sparging. A mixture of these two gases in varying content is also used in some cases. Among the three gases, nitrogen has been widely used in wineries for sparging purpose because of its low solubility (eight to nine times) than carbon dioxide and low specific gravity. The solubility of N_2 in water at atmospheric pressure and at 20°C is about 19 mg/L.

CO_2 is released during the fermentation. Its solubility in water at atmospheric pressure and at 20°C is about 1.69 g/L. It is heavier than air with a density at 0°C of 1.52 as compared to the density (1.0) of air. Dissolved CO_2 gives an indication of tartness and freshness to a wine. It improves palatability and enhances the flavor of the wine. Normally, white wines are developed with higher CO_2 levels than red wines. N_2 sparging declines the level of O_2 to below 1% under its permissible perimeter and prevents the wine from oxidation. During transfer, bottling, wine storage with ullage space, wine faces the risk of getting oxidized with oxygen.

5.7.3 Aging

Aging of wine and its capacity to improve wine quality and flavor, distinguishes it from most other consumable goods. Only some wines have the capability to considerably improve with age. After fermentation and clearing, wine needs to be aged. Aging can be done by storing the wine in wooden or the wine bottle. Aging is done in a wooden vat for least 6 months in most red wines to add the flavor of the wood to the wine (Wimalsiri et al., 1971). Red wines are commonly matured in oak barrels and this is thought to assist with stabilization of color. The nature of the handling operations of wines in barrels means that they are intermittently mildly aerated, and this can lead to formation of acetaldehyde bridged polymeric pigments (Ribéreau-Gayon et al., 1983). All wines treated with oak showed a trend toward slightly higher hue values than the unoaked wines.

Ratio of sugars, acids, and phenolics to water determines the aging of wine. The less the water content in the grapes prior to harvest, the more likely the resulting wine will have aging potential. Grape variety, climate, vintage, and viticultural practices also plays important role to determine aging potential. Grape cultivars with thicker skins, from a dry growing season and yields will have less water content and a higher ratio of sugar, acids, and phenolics (Robinson, 2003). Aged red wines acquire significantly diverse polyphenolic composition compared with young wines, largely not only due to the formation of polymeric compounds but also because of hydrolysis, oxidation, and other transformations that occur in inhabitant grape phenolics during aging (Arnous et al., 2001).

In general, wines having low pH (like Sangiovese and Pinot noir) have a better capability of aging. White wines have better aging potential because of high acidity. Acidity in white wines plays a similar role that tannins play in red wines as a preservative for longer aging and storage. The process of developing white wines includes little to no skin contact, which results in white wines having a significantly lower content of phenolic compounds, though fermentation carried out in oak barrels and oak aging can impart some phenolics. While the red wines have a high level of flavor compounds, such as phenolics that increase the likelihood that a wine will be able to age. Cabernet Sauvignon, Nebbiolo, and Syrah or Shiraz have high levels of phenolics (Robinson, 2006). Aging in wooden barrels improved the wine quality by adding desirable components including ethyl acetate, phenolics, etc. and led to the reduction in undesirable components such as *n*-proponal, *n*-butanol, iso-butanol, and isoamyl alcohols (Soni et al., 2009). The storage of amla wine in oak wooden barrels for a month improves the quality and sensory attributes than the wine stored in glass bottles (Soni et al., 2009).

Red (Punjab MACS purple syn. H-516) and white (H-144) wines prepared under the optimized prefermentative, fermentative, and malolactic parameters were stored in amber colored glass bottles at 15°C for the debris and other solid particles to settle. The siphoning was repeated after every 15 days till there was no further settling of debris. No settling or fining agent was added. Finally, cleared wine is filled in the colored bottles and nitrogen gas is sparged in the ullage space at 1.5 bar pressure for 3–5 s and bottles were capped with oak corks immediately after sparging.

5.8 Shelf-Life Study

Stored wine of both cultivars was analyzed for various physicochemical parameters at different periods of time for up to 12 months. Ethanol content in the wines was nonsignificantly decreased (12.0–11.7%v/v in Punjab MACS purple and 11.2–11.0% v/v in H-144) during the storage period due to nitrogen sparging and low ullage head space. Bottles capped with oak corks during the storage time showed the significant increase in the total phenols content as the ethanol in the wine extracts the phenolics from the oak wood cap to improve the aroma, taste as well as antioxidants content of the wine. Amla wine matured in oak wood barrel had high quantity of desirable components including ethyl acetate and phenolics, etc. and was better in its sensory characteristics than the wine stored in glass bottles (Soni et al., 2009). Decrease in anthocyanins and pH was also found to be nonsignificant during the storage period while decrease in the ascorbic acid was significant in both the wines. While Kallithraka et al. (2009) observed a decrease in total phenols up to 6 months of storage and an increase thereafter, Ivanova et al. (2009) in their study observed a continuous decrease up to 16 months of storage. Literature reveals that fruit rapidly losses ascorbic acid (21%–83%) in 4–8 weeks but it is retained to a large extent in wines. Such observations have been made by Dlamini and Dube (2008) where they reported 72% retention of ascorbic acid in Marula wine. This retention of ascorbic acid in white wines is a good sign for wines as it prevents wine browning during storage and also decreases in phenolic compounds (Shiri et al., 2011). Further ascorbic acid is a known antioxidant as it quenches free radicals otherwise harmful to health. Our results showed that the yeast was undetectable after 60 days of storage in both varieties while the bacterial count on MRS during the storage period showed that bacteria was undetectable after 30 days. This was accredited to the absence of sugars and nutrients in wines coupled with repeated racking and nitrogen sparging. Hence, it can be concluded that wine prepared from grapes can be stored with all desirable characteristics for at least 1 year (the period studied) with inert nitrogen sparging and low ullage space in colored bottles (Table 5.2).

5.9 Organoleptic Analysis

Red and white wine samples stored in amber colored bottles (nitrogen sparged and oak corked) at 15°C were subjected to organoleptic analysis (amino acids, glycerol, anthocyanins, ascorbic acid, total phenolics, and total flavonoids). Qualitative study of red and white wines with respect to amino acids carried out by Thin Layer Chromatography showed spots at distances of 2.7, 4.3, 6.1, 8.8, and 10.2 cm, showing R_f value of 0.17, 0.24, 0.29, 0.41, 0.59, and 0.79, respectively. Based on the comparison available in literature (tera.chem.ut.ee; www.reachdevices.com), the R_f values were designated to the presence of histidine or arginine, proline, glycine, isoleucine or tryptophan, and leucine amino acids in the both wines. It was reported that amino acid profile of the grape berries depends on the geographic conditions, climatic conditions, cultivars, and nitrogen supply during growth of berries as lowering of nitrogen supply reduced free amino acid contents affecting arginine the most, identified in "Pinot noir" berries (Lee and Schreiner, 2010). Bouloumpasi et al. (2002) studied the amino acid profile of 50 Greek red wines and reported that L-glutamic acid and L-arginine were predominant over other amino acids. Other literature revealed that arginine and leucine amino acids are present in high amounts followed by isoleucine and glycine in both grapes and wines (Georgiev et al., 2014). Glycerol contributes to the perceived viscosity and fullness of wines. Its content was found to be in the range of 5.1–6.9 g/L in fresh wines samples while it increased during the aging of wine to a maximum of 8.2 g/L in red wine. Gawel et al. (2007) reported that glycerol content was increased during the storage period and increased up to 9.9 g/L in Australian white table wines and 9.36 g/L in South African dry white wines.

Table 5.2 Effect of Storage Time Physicochemical Properties of Wine cv. Punjab MACS Purple and H-144

Storage Time (Days)	Parameters									
	Ethanol (% v/v)		Anthocyanins (mg/100 mL)		Ascorbic Acid (mg/100 mL)		Total Phenols (mg/100 mL)		pH	
	Punjab MACS Purple	H-144	Punjab MACS Purple	H-144	Punjab MACS Purple	H-144	Punjab MACS Purple	H-144	Punjab MACS Purple	H-144
0	12.0	11.2	39.8	1.76	1378	1241	297.8	156.9	3.4	3.4
15	12.0	11.2	39.4	1.72	1311	1224	296.2	153.1	3.4	3.4
30	12.0	11.2	39.5	1.70	1288	1203	303.5	158.9	3.4	3.4
60	12.0	11.2	39.4	1.39	1285	1167	341.4	209.2	3.3	3.3
90	12.0	11.1	39.2	1.12	1276	1165	382.9	248.1	3.4	3.2
120	12.0	11.1	38.8	1.15	1278	1143	382.2	258.3	3.3	3.3
150	11.9	11.2	38.2	1.12	1267	1132	380.4	257.7	3.4	3.3
180	11.9	11.1	38.4	1.10	1266	1136	392.8	253.2	3.3	3.2
210	11.8	11.0	38.3	0.97	1246	1134	389.2	255.1	3.4	3.2
240	11.9	11.1	38.2	0.94	1247	1133	389.0	249.0	3.3	3.3
270	11.7	11.1	38.2	0.96	1234	1129	382.8	251.8	3.3	3.2
300	11.7	10.9	38.3	0.96	1237	1128	383.5	250.8	3.4	3.3
330	11.6	11.0	38.3	0.97	1236	1127	383.3	252.4	3.4	3.3
360	11.7	11.0	38.3	0.98	1237	1129	382.4	251.7	3.4	3.3
CD (5%)	NS	NS	NS	NS	0.358	0.417	0.287	0.241	NS	NS

It has been observed that wine bottles corked with oak corks during storage showed an increase in total phenolic and total flavonoid contents. Similarly, Majdak et al. (2002) studied the total polyphenols and various aroma compounds in wine during the storage in oak barrels that showed the presence of 11 phenolics including vanillic acid, gallic acid, caffeic acid, *p*-coumaric acid, protocatehic acid, *p*-hydroxybenzoic acid, chlorogenic acid, ferulic acid, siringic acid, gentisic acid, and sinapinic acid. Antioxidant assay of Punjab MACS purple and H-144 by FRAP method revealed that red wine had high antioxidant potential of the order of 54.76 GAE/L than white wine (32.65 GAE/L). However, both wines hold good antioxidant potential that add to their nutritive value. In literature, total phenolic and total flavonoid contents in wines studied by Mitic et al. (2010) ranged from 238.3 to 420.6 mg gallic acid equivalents per L and from 42.64 to 81.32 mg catechin equivalents per L of wines, respectively. A high and significant correlation between antioxidant activity and total phenolic content was observed in wines ($R^2 = 0.968$, $P < 0.01$).

5.10 Sensory Analysis

Final objective of any consumable product is its consumer's acceptability. A good taste united with nutrition attracts the consumer. Wine, a highly nutritive product, is always subjected to sensory analysis by a panel of trained panelists to check the acceptability of the final product. Wine sensory evaluation and analysis plays a major role in the way people perceive wine. It is important to understand consumer preferences in order to produce wines that will be sold successfully in the wine market. Sensory analysis of the wine is based upon following aspects: vision, smell, and taste. Human aspects control in terms of sensory evaluation is one of the most complicated factors of sensory evaluation that can be accomplished by carefully selecting the people who will be participating in the tasting wine. Significant qualities in a sensory panelist include availability, interest, dependability, objectivity, and acute senses of taste and smell (Zoecklein, 2005). Panelists can be classified as consumers, experienced/trained, and semi-trained. A pool of 12 or more trained or semi-trained panelists are required for testing the wines at laboratory scale level. Trained judges are instructed about the wine characteristics of the interest so that they can assess wines for specific characteristic and they are trained to access the qualitative and quantitative differences between samples. Different Davis score cards (20–80 point scale) are available to rate the wines. Nowadays, a modified Davis score card (Amerine and Roessler, 1976) has been used for sensory analysis that evaluates the wine aspects on 80-point hedonic scale.

5.11 Conclusion

The chapter comprises a complete evaluation of different prefermentation, fermentation MLF, and postfermentation parameters using a statistical software "Design Expert." The use of statistical software in the efficient planning of the experiment and analyzing the results taking 3D graphs into consideration that adds quality to work and help in determining the optimum results with confidence. Hence, the study concluded the selection regarding the optimum maturity stage of grape cultivars, optimization of prefermentation, fermentation, and postfermentation parameters to develop an efficient processing technology (Figs. 5.1 and 5.2) to produce good quality local grape wines.

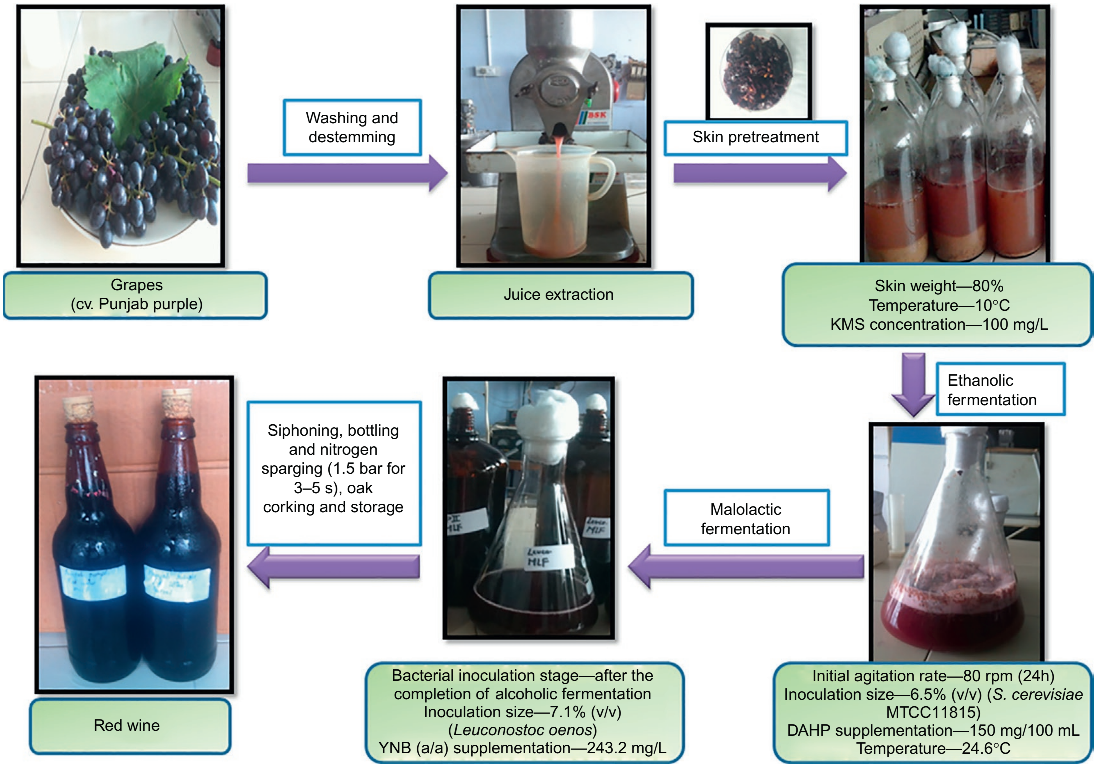
Grapes
(cv. Punjab purple)
Washing and destemming
Juice extraction
Skin pretreatment
Skin weight—80%
Temperature—10°C
KMS concentration—100 mg/L
Ethanolic fermentation
Initial agitation rate—80 rpm (24h)
Inoculation size—6.5% (v/v) (*S. cerevisiae* MTCC11815)
DAHP supplementation—150 mg/100 mL
Temperature—24.6°C
Malolactic fermentation
Bacterial inoculation stage—after the completion of alcoholic fermentation
Inoculation size—7.1% (v/v)
(*Leuconostoc oenos*)
YNB (a/a) supplementation—243.2 mg/L
Siphoning, bottling and nitrogen sparging (1.5 bar for 3–5 s), oak corking and storage
Red wine

Fig. 5.1 Flow diagram for production of red wine (cv. Punjub purple syn. H-516).

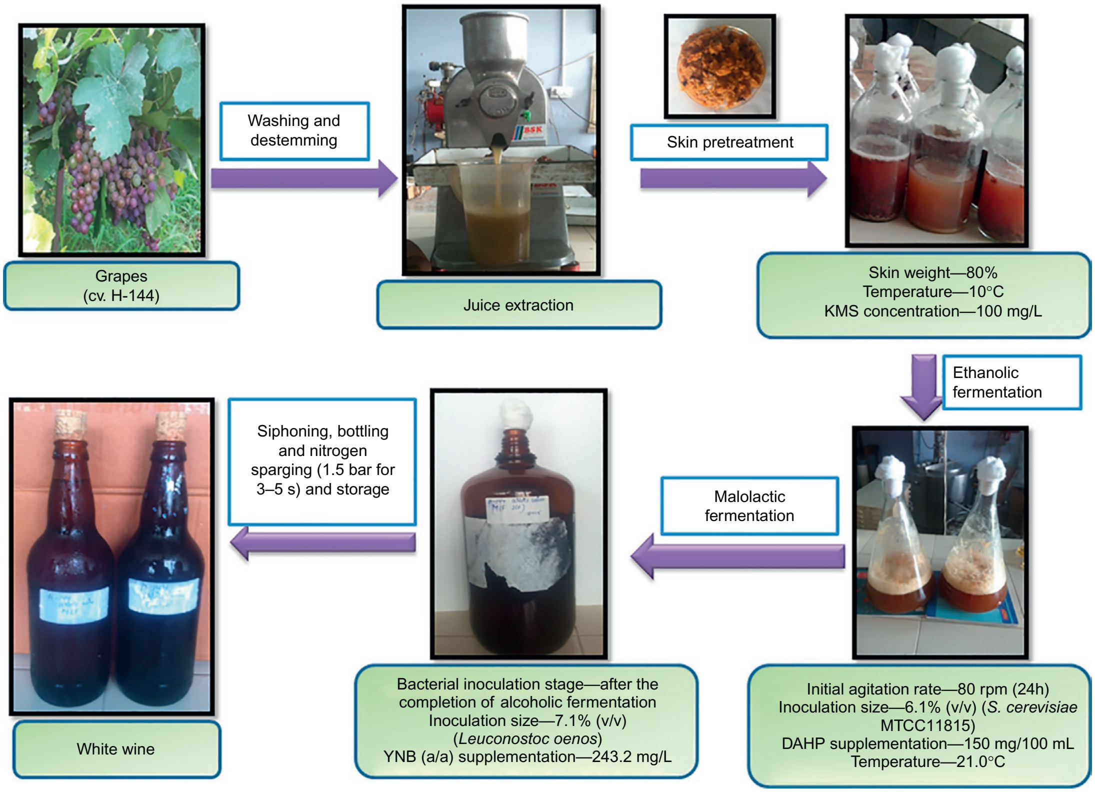
Grapes
(cv. H-144)
Washing and destemming
Juice extraction
Skin pretreatment
Skin weight—80%
Temperature—10°C
KMS concentration—100 mg/L
Ethanolic fermentation
Initial agitation rate—80 rpm (24h)
Inoculation size—6.1% (v/v) (*S. cerevisiae* MTCC11815)
DAHP supplementation—150 mg/100 mL
Temperature—21.0°C
Malolactic fermentation
Bacterial inoculation stage—after the completion of alcoholic fermentation
Inoculation size—7.1% (v/v)
(*Leuconostoc oenos*)
YNB (a/a) supplementation—243.2 mg/L
Siphoning, bottling and nitrogen sparging (1.5 bar for 3–5 s) and storage
White wine

Fig. 5.2 Flow diagram for production of white wine (cv. H-144).

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6

AN INFLUENCE OF DIFFERENT YEAST SPECIES ON WINE AROMA COMPOSITION

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Abbreviations

C. molischiana	Candida molischiana
C. pulcherrima	*Candida pulcherrima*
C. stelatta	*Candida stellata*
C. wickerhamii	*Candida wickerhamii*
C. zemplenina	*Candida zemplenina*
D. pseudopolymorphus	*Debaryomyces pseudopolymorphus*
D. variji	*Debaryomyces variji*
H. anomala	*Hanseniaspora anomala*
H. guilliermondii	*Hanseniaspora guilliermondii*
H. osmophila	*Hanseniaspora osmophila*
H. uvarum	*Hanseniaspora uvarum*
H. vineae	*Hanseniaspora vineae*
I. orientalis	*Issatchenkia orientalis*
I. terricola	*Issatchenkia terricola*
K. aerobia	*Kazachstania aerobia*
K. gamospora	*Kazachstania gamospora*
K. lactis	*Kluyveromyces lactis*
K. marxianus	*Kluyveromyces marxianus*
K. thermotolerans	*Kluyveromyces thermotolerans*
L. thermotolerans	*Lachancea thermotolerans*
M. pulcherrima	*Metschnikowia pulcherrima*
P. anomala	*Pichia anomala*
P. ferments	*Pichia fermentas*
P. kluyveri	*Pichia kluyveri*
P. membranifaciens	*Pichia membranifaciens*
S. bacillaris	*Starmerella bacillaris*
S. cerevisiae	*Saccharomyces cerevisiae*
S. paradoxus	*Saccharomyces paradoxus*
S. pombe	*Schizosaccharomyces pombe*
S. uvarum	*Saccharomyces uvarum*
T. delbrueckii	*Torulaspora delbrueckii*
W. anomalus	*Wickerhamomyces anomalus*

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W. saturnus	*Williopsis saturnus*
Z. bailii	*Zygosaccharomyces bailii*
Z. kombuchaensis	*Zygosaccharomyces kombuchaensis*

6.1 Introduction

Wine, with more than 6000 years old background, is one of the world's oldest fermentation beverage. Traditionally speaking, wine fermentation is a natural, spontaneous process carried out by indigenous yeasts mainly present in grapes. But wine fermentation is also a unique microbiological process, crucial for final wine chemical composition and quality, strongly influenced by fermentation conditions and yeast species used.

Wine is a complex mixture of different classes of compounds present in a wide range of concentrations. Till date over 1300 chemical compounds have been identified in wine; most of which are present as a result of the yeast metabolism during fermentation (Ebeler, 2001). Researchers related the interactions between yeast and grape chemical compounds have revealed an essential role of yeast contributing to the appearance, aroma, flavor, and structure of the wine. According to Fleet (2003), yeast influences wine aroma in several ways among them the most important is the alcoholic fermentation of the grape juice, de novo biosynthesis of aroma compounds, and transformation of flavor-neutral grape compounds into active once. Also among them, fermentation-derivated volatiles including esters, higher alcohols, and volatile acids as well as varietal compounds such as thiols and terpenes are the most abundant in total aroma composition of wine (Lambrechts and Pretorius, 2000).

Modern winemaking has been found on the use of selected strains of *Saccharomyces cerevisiae*, due to its advantages of pure and complete fermentation coupled with good flavor characteristics as well as the high alcohol conversion rate. Today, there are numerous—perhaps hundreds of—diverse yeast strains available, and the winemaker's choice can substantially effect the quality of the wine (Swiegers et al., 2005). Albeit *S. cerevisiae* is the yeast primarily responsible for alcoholic fermentation, it is not the only the yeast species found in grapes. In fact, at the time of harvest, *S. cerevisiae* typically accounts for 1% or less of the total yeast population found in grape berries (Barata et al., 2012). There are currently over 20 different yeast genera such as *Saccharomycodes*, *Schizosaccharomyces*, *Candida*, *Torulaspora*, *Debaryomyces*, *Issatchenkia*, *Pichia*, *Kluyveromyces*, *Metschnikowia*, *Hanseniaspora (Kloeckera)*, *Rhodotorula*, *Brettanomyces (Dekkera)*, and *Zygosaccharomyces* thought to be associated with grapes and wine (Kurtzman and Fell, 2011). Although in the past non-*Saccharomyces* yeasts were considered detrimental (or at the very least inconsequential) to the winemaking process (Dubourdieu et al., 2006; Bartowsky and Pretorius, 2009), nowdays many authors (Romano et al., 2003; Comitini et al., 2011; Jolly et al., 2014; Benito et al., 2016a) pointed out that the presence of specific non-*Saccharomyces* yeasts during fermentation can, under the right circumstances, contribute significantly to the definite flavor and quality of wine. So recent trends in enology have led to the commercial production and application of non-*Saccharomyces* yeasts in winemaking. But most non-*Saccharomyces* yeasts are not able to consume all sugar present in grape must and therefore need to be used as part of sequential inoculation, mixed, or multistarter cultures with *S. cerevisiae* strains (Azzolini et al., 2015; Belda et al., 2017).

6.2 Primary (Varietal) Wine Aroma

The distinctive aroma of the wine is determined by many factors, including grape variety, viticultural and winemaking practices, wine maturation, and storage conditions. Compounds contributing to wine quality and aroma can be found in free and bound form (Hjelmeland and Ebeler, 2015). So the odorant ones are produced or released from nonvolatile compounds or their precursors by the yeasts during fermentation and aging of wine. A recent report has shown that more than 40 different aroma chemicals belonging to different chemical classes are formed or released from precursors during fermentation by *S. cerevisiae* (Loscos et al., 2007). Results by Hernandez-Orte et al. (2008) also confirmed that large pools of aroma compounds, many of which have been previously identified as essential wine odorants, are formed from nonaromatic grape precursors by the action of yeasts belonging to entirely different genera. Many of these compounds are already present in the grape and come through fermentation unaltered or with only minor modifications. All these, including main thiols, terpenes, and C_{13}-norisoprenoids are responsible for the primary aroma of wines.

6.2.1 Yeast Influence on Varietal Thiol Composition

It is well known that great majority of sulfur compounds have a negative effect on wine sensory characteristics. Based on the chemical structure they can be divided into sulfides, sulfur-containing fusel alcohols, and thiols. Among the above-mentioned classes of sulfur compounds, thiols positively contribute to wine aromas. The volatile thiols 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexane-1-ol (3MH), and 3-mercapto-hexyl acetate (3MHA) have great importance to wine flavor. The volatile thiols are nonexistent in grape. Many studies have confirmed that grape contains 4MMP and 3MH in nonvolatile forms. These nonvolatile forms are composed of 4-MMP or 3MH bound to the glutathione or cysteine. Cysteinylated precursors occur at higher levels in grapes and then glutathionylated precursors. In wine, the release of 4MMP and 3MH can be explained by three biogenesis pathways (Fig. 6.1). The first pathway includes cysteinylated precursors, *S*-3-(hexane-1-ol)-cysteine, and *S*-3-(mercapto-4-methylpentan-2-one)-cysteine. Yeast β-lyase cleave cysteine unit and release 4MMP and 3MH. The second biogenesis pathway involves the glutathionylated precursors: *S*-3-(hexane-1-ol)-glutathione and *S*-3-(mercapto-4-methylpentan-2-one)-glutathione and activity of yeast β-lyase. The third pathway concerns C_6 unsaturated compounds, such as (*E*)-2-hexenal. During the fermentation addition of sulfur on (*E*)-2-hexenal occurs. The biogenesis pathway of 3MHA is quite different, yeast ester-forming alcohol acetyltransferase converts 3MH to 3MHA (Peyrot Des Gachons et al., 2000; Fedrizzi et al., 2009; Tominaga et al., 1998; Swiegers and Pretorius, 2007; Schneider et al., 2006; Roland et al., 2011).

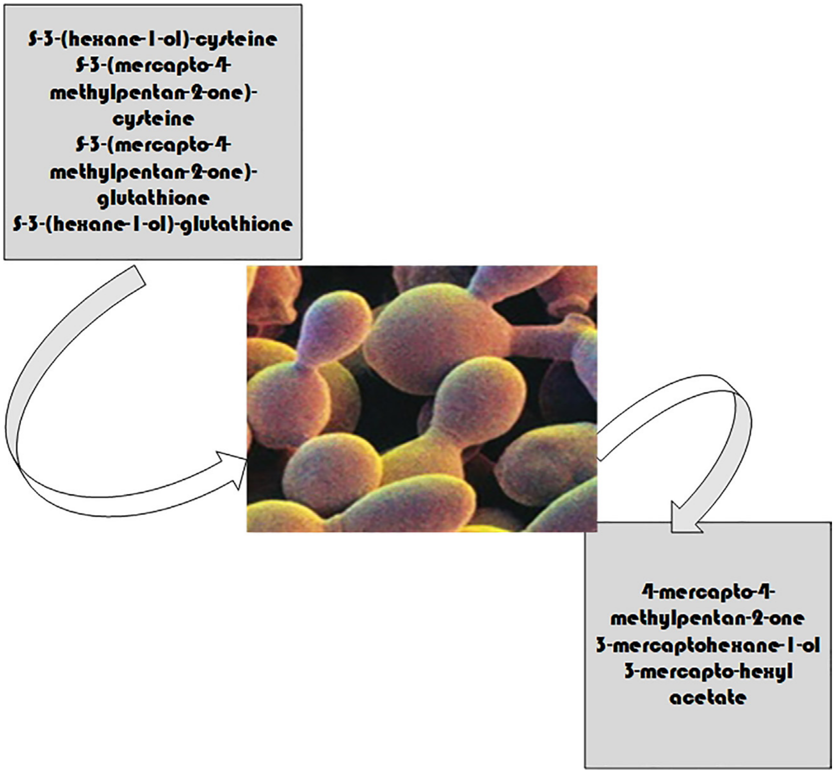
S-3-(hexane-1-ol)-cysteine
S-3-(mercapto-4-methylpentan-2-one)-cysteine
S-3-(mercapto-4-methylpentan-2-one)-glutathione
S-3-(hexane-1-ol)-glutathione
4-mercapto-4-methylpentan-2-one
3-mercaptohexane-1-ol
3-mercapto-hexyl acetate

Fig. 6.1 Schematic representation release of volatile thiols by non-*Saccharomyces* yeast.

Sulfur-containing compounds such as 3-mercaptohexan-1-ol (3MH), its acetylated derivative 3-mercaptohexyl acetate (3MHA), and methyl-4-sulfanylpentan-2-one (4MSP) are mostly responsible for the "box tree," "grapefruit," "guava," and "passion fruit" tropical aromas in wine, primarily from Sauvignon blanc grapes but also in wines made from Gewürztraminer, Riesling, Colombard, Petit manseng, Cabernet Sauvignon, and Merlot. Their strong influence is due to their low detection thresholds ranging from 3 ng/L (4MSP), 60 ng/L (3MH) to 4 ng/L (3MHA). In Sauvignon Blanc wines analyzed from Bordeaux and Sancerre, the 4 MSP concentration ranged from 4 to 24 ng/L the 3MH concentration from 733 to 12,822 ng/L and the 3MHA concentration from 212 to 724 ng/L indicating their important contribution to wine aroma (Dubourdieu et al., 2006). They exist as nonvolatile *S*-cysteine conjugate precursors in the must, and like terpenes, yeast is able to release these bound compounds during fermentation (Anfang et al., 2009; Swiegers et al., 2009; Zott et al., 2011). It has been suggested that the cysteine conjugates themselves are breakdown products of glutathionyl conjugates what was confirmed by Subileau et al. (2008) who demonstrated that the glutathionyl conjugate, and not the cysteine conjugate, is the major precursor of 3MHA. Current knowledge has shown a marked difference between *S. cerevisiae* and non-*S. cerevisiae* strains ability to release thiol precursors available in grapes. So according to Coetzee and du Toit (2012), most *S. cerevisiae* strains are only able to release around 10% of the thiols mainly because of low enzyme affinity for complex cysteinylated substrates. On the contrary, investigation results of Swiegers et al. (2009) indicated that the *S. cerevisiae* yeast strains varied significantly regarding their capabilities to produce volatile thiols. Several different commercial *S. cerevisiae* strains have been appraised for their capability to liberate varietal thiols. While differences have been found for the 4MSP release, no significant changes have been found for 3MH release (Murat et al., 2001). King et al. (2008) investigating the effect of coinoculating *S. cerevisiae* strains on the thiol composition found out that 3SH and 3SHA increased in the Vin7/QA23 coinoculation compared to single-strain used. Among the non-*Saccharomyces* yeasts, some researchers (Zott et al., 2011; Sadoudi et al., 2012) have investigated the impact of *Torulaspora delbrueckii* yeast metabolism on the volatile thiol formation in pure and mixed cultures. According to them, pure *T. delbrueckii* had a good capacity of releasing 3SH but poor capacity to form 4MSP. Also in a simultaneously mixed culture (*T. delbrueckii*/*S. cerevisiae*) at ratio 10:1 a decrease in 3SH and 3SHA was noticed, compared to pure *S. cerevisiae* yeast. Results by Renault et al. (2016) pointed out the poor property of *T. delbrueckii* to produce 4MSP and 3SHA but good release capacity of 3SH in pure as well as in sequential *(T. delbrueckii*/*S. cerevisiae*) fermentations. According to Renault et al. (2016), *T. delbrueckii* only assimilated the glutathionylated precursor, while both precursors (cysteinylated and glutathionylated) forms were metabolized by *S. cerevisiae*. In sequential fermentation stronger *T. delbrueckii* development and synergistic interaction with *S. cerevisiae* caused an enhanced 3SH and 3SHA production compared to pure *S. cerevisiae* yeast. Partially contradictory results were published by Belda et al. (2017) who indicated an important impact of *T. delbrueckii* strain in aroma complexity of white wines achieving increased concentrations not only of 3SH but also 4MSP showing that some *T. delbrueckii* strains could be able to assimilate the cysteinylated precursors of thiol aromas. Among other non-*Saccharomyces* species, Anfang et al. (2009) showed the potential impact of *Candida zemplinina* (synonym *Starmerella bacillaris*) and *Pichia kluyveri* (synonym *Candida stellata*) on the release of 3SH and its acetate and that cofermentations using *P. kluyveri* with *S. cerevisiae* may provide a way of increasing 3SHA concentrations in New Zealand Sauvignon Blanc. But the elevation in thiols concentration was only seen in coferments with certain *S. cerevisiae* strains suggesting that the nature of this interaction may not be generalized to the species level. Zott et al. (2011) evaluated 15 non-*Saccharomyces* strains from seven species (*Kluyveromyces thermotolerans*, *Issatchenkia terricola*, *Issatchenkia orientalis*, *Candida zemplinina*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Torulaspora. delbrueckii*) together with two *S. cerevisiae* commercial strains, Zymaflore X5 and Zymaflore F10. According to Masneuf-Pomarède et al. (2006), Zymaflore X5 showed high ability to liberate volatile thiols. Achived results pointed out that non-*Saccharomyces* strains tested, compared to *S. cerevisiae* strains were able to release equivalent or higher amounts of 3SH from its synthetic or natural precursor, except *I. orientalis*, *C. zemplinina*, and *H. uvarum*. Only *T. delbrueckii*, *M. pulcherrima*, and *Lachancea thermotolerans* had a high capacity to release 3SH from its precursor form while *M. pulcherrima*, *H. uvarum*, and *L. thermotolerans* were able to produce larger amounts of 4MSP. As demonstrated for *S. cerevisiae* species (Swiegers et al., 2009), the influence of non-*Saccharomyces* strains tested can also have the strong effect on volatile thiol release. The strain effect may explain the contrasting results between the study by Zott et al. (2011) and that of Anfang et al. (2009) concerning *C. zemplinina* ability of liberating thiol compounds. All these data confirm the unexplored potential of yeast species in increasing wine flavor complexity and their application in winemaking (Table 6.1).

6.2.2 Yeast Influence on Varietal Terpenes Composition

Terpenes are secondary plant metabolites composed of isoprene (C_5H_8) as a basic unit. Based on the number of isoprene units link

Table 6.1 Dependence of Volatile Thiols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast

Yeast	4MMP	3MH	3MHA	Refs.
S. cerevisiae	3	380	20	Swiegers et al. (2009)
S. cerevisiae	3	280	60	Swiegers et al. (2009)
S. cerevisiae	3	290	65	Swiegers et al. (2009)
S. cerevisiae	6	500	30	Swiegers et al. (2009)
S. cerevisiae	14	450	80	Swiegers et al. (2009)
S. cerevisiae	4.5	300	55	Swiegers et al. (2009)
S. cerevisiae	2.5	380	30	Swiegers et al. (2009)
S. cerevisiae	na	610	80	Belda et al. (2017)
S. cerevisiae	70	780	80	Belda et al. (2017)
T. delbrueckii + *S. cerevisiae*	820	780	80	Belda et al. (2017)
T. delbrueckii +*S. cerevisiae*	1100	810	80	Belda et al. (2017)
S. cerevisiae	30.2	3064.4	13.6	Sadoudi et al. (2012)
M. pulcherrima	27.8	3151.8	3.6	Sadoudi et al. (2012)
T. delbrueckii	7.1	2388.3	13.0	Sadoudi et al. (2012)
C. zemplinina	44.0	2075.9	10.3	Sadoudi et al. (2012)
C. zemplinina + *S. cerevisiae*	10.2	2121.4	31.6	Sadoudi et al. (2012)
M. pulcherrima + *S. cerevisiae*	7.0	1648.2	125.4	Sadoudi et al. (2012)
T. delbruecki + *S. cerevisiae*	11.4	2205.7	26.3	Sadoudi et al. (2012)
T. delbrueckii	na	623	14	Renault et al. (2016)
T. delbrueckii + *S. cerevisiae* sequential	na	1312	218	Renault et al. (2016)
T. debrueckii + *S. cerevisiae* simultaneous	na	362	79	Renault et al. (2016)
S. cerevisiae	na	303	83	Renault et al. (2016)
S. cerevisiae	na	222	114	Masneuf-Pomarède et al. (2006)
S. cerevisiae	na	1664	479	Masneuf-Pomarède et al. (2006)
S. cerevisiae	na	392	175	Masneuf-Pomarède et al. (2006)
S. cerevisiae	5	243	112	Masneuf-Pomarède et al. (2006)
S. cerevisiae	9	312	56	Masneuf-Pomarède et al. (2006)
S. cerevisiae	17	311	79	Masneuf-Pomarède et al. (2006)
S. cerevisiae	17	289	46	Masneuf-Pomarède et al. (2006)
S. cerevisiae	23	200	57	Masneuf-Pomarède et al. (2006)
P. kluyveri + *S. cerevisiae*	na	1100	1000	Anfang et al. (2009)
P. kluyveri + *S. cerevisiae*	na	500	450	Anfang et al. (2009)
P. kluyveri + *S. cerevisiae*	na	1300	1200	Anfang et al. (2009)

Continued

Table 6.1 Dependence of Volatile Thiols Concentration Upon Saccharomyces and Non-Saccharomices Yeast—cont'd

Yeast	4MMP	3MH	3MHA	Refs.
P. kluyveri + *S. cerevisiae*	na	780	800	Anfang et al. (2009)
P. kluyveri + *S. cerevisiae*	na	550	780	Anfang et al. (2009)
P. kluyveri + *S. cerevisiae*	na	550	600	Anfang et al. (2009)
S. cerevisiae	na	900	400	Anfang et al. (2009)
S. cerevisiae	na	1200	580	Anfang et al. (2009)
S. cerevisiae	na	1500	600	Anfang et al. (2009)
S. cerevisiae	na	850	370	Anfang et al. (2009)
S. cerevisiae	na	1300	590	Anfang et al. (2009)
S. cerevisiae	na	1600	610	Anfang et al. (2009)
P. kluyveri	na	40	620	Anfang et al. (2009)
S. cerevisiae	2.8	800	40	King et al. (2008)
S. cerevisiae	1.6	700	45	King et al. (2008)
S. cerevisiae	3.0	1200	na	King et al. (2008)
S cerevisiae	2.3	1000	60	King et al. (2008)
S. cerevisiae	na	1200	25	King et al. (2008)

Results are expressed in μg/L.
na, not analyzed.

together, this large class of compounds can be divided into hemiterpenes (single isoprene unit), monoterpenes (two isoprene unit), sesquiterpenes (three isoprene unit), etc. In grape and wine, the most abundant are monoterpenes. Grape almost exclusively contains glycosidic forms of monoterpenes which can be considered as nonvolatile precursors of wine terpenes. Terpenes such as linalool, nerol, geraniol, α-terpineol, and citronellol are the most common aglycons. In grape, the aglycon moiety is covalently bound to the following disaccharides:6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose, 6-*O*-α-L-ramnopyranosyl-β-D-glucopyranose, and 6-*O*-β-L-apiofuranosyl-β-D-glucopyranose. Throughout the vinification, the release of bound terpenes may occur. This process catalyzed glycosidase enzymes (Fig. 6.2). Enzyme hydrolysis of monoterpenes involves two stages. In the first stage, an α-L-arabinofuranosidasa, a β-L-apiofuranosidasa or an α-L-ramnopyranosidasa cleave the 1,6-glycosidic bond. While in the second stage by the action of a β-D-glucopyranosidase monoterpenes are liberated from the monoterpenyl-β-D-glucopyranose

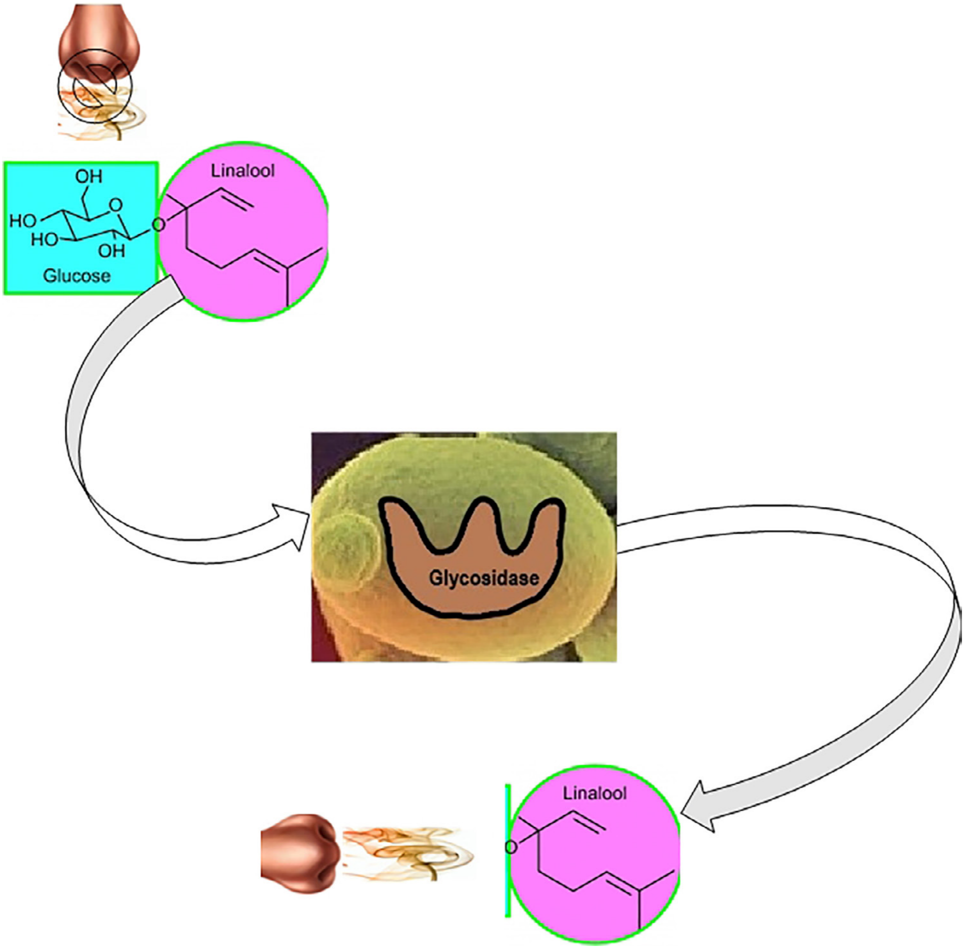

Fig. 6.2 Schematic representation of enzyme cleveage of linalool-3-*O*-glucoside in non-*Saccharomyces* yeast.

(Maicas and Mateo, 2005; Swiegers et al., 2005). Under certain conditions, some yeast species can synthesize monoterpenes. These monoterpenes are derived from sterol biosynthetic pathway. One of the key reaction in this pathway catalyzed by geranyl-PP synthase is the formation of geranyl-PP from isopentyl-PP. By different chemical or enzymatic reaction final monoterpenes (geraniol, nerol, citronellol, and α-terpineol) are formed. Synthesis monoteprenes can occur via the leucin-mevalonic acid as well (Ugliano and Henschenke, 2009; Carrau et al., 2005). In some case, citronellol can be formed by yeast-catalyzed reduction of free geraniol (Ugliano et al., 2006).

The majority of identified 50 terpenes that contribute to grape and wine flavor and aroma belong to the monoterpenes (monoterpenoids) subfamily, while rotundone is the only sesquiterpene subfamily member reported to be important for wine aroma and flavor (Dunlevy et al., 2009). The most odoriferous are monoterpene alcohols, notably linalool, α-terpineol, nerol, geraniol, citronellol, and hotrienol with characteristic floral, fruity and citrus aroma, and low olfactory perception thresholds between 18 and 400 μg/L. Among them linalool is one of the most significant monoterpenes, being present at levels higher than its perception threshold of 50 μg/L (Ribéreau-Gayon et al., 2006). The flavor threshold of α-terpineol is much higher than that of linalool (400 μg/L) while for linalool oxides is 6000 μg/L (Fariña et al., 2015). Most grape varieties contain free and bound glycoside terpenes. In general, in grape must monoterpenoid compounds in the bound form are mostly sugar-bound conjugates (glucose or other sugars) and they are usually more abundant than the free ones (Mateo and Jiménez, 2000). Their liberation during fermentation is closely related to yeast β-glycosidases activity (Ugliano et al., 2006). Already Grossmann et al. (1987) reported β-glycosidases activity in *Hansenula* sp. strain isolated from fermenting must while Gondé et al. (1985) and Leclerc et al. (1984) noticed β-glycosidases activity in *Candida molischiana* and *Candida wickerhamii* yeast strains. The ability of these yeasts and their enzymes to hydrolyze grape monoterpenyl glycosides and their use in winemaking was reported in the work by Gunata et al. (1990). Many types of research have shown that unlike *S. cerevisiae*, non-*Saccharomyce*s yeasts can release odourless grape precursors through their β-glucosidase activity, biotransform them or even synthesize new aroma molecules that can enhance wine varietal aroma (Hernandez-Orte et al., 2008; Jolly et al., 2014). The work by Furdíková et al. (2014) evaluated three selected autochthonous and one commercial *S. cerevisiae* strains. Based on the results one of the strains used considerably distinguished from the others thanks to the higher *cis*-rose oxide production ability. On the contrary, no significant influence of 13 *S. cerevisiae* commercial yeast strains tested on the liberation of some monoterpenes like *cis* and *trans* linalool oxide and α-terpineol was noted, the only exception was the liberation of linalool (Srisamatthakarn, 2011). Englezos et al. (2015) tested 63 different strains and found that only 5% of the isolates showed β-glucosidase activity indicating a large amount of metabolic diversity within the species. Potential of *Debaryomyces pseudopolymorphus* β-glucosidase activity as a wine aroma-enhancing enzyme was confirmed by the work of Cordero Otero et al. (2003) who noticed significant increase in the concentrations of free volatiles (citronellol, nerol, and geraniol) during the fermentation of Chardonnay juice inoculated with both *D. pseudopolymorphus* and a widely used commercial starter culture strain of *Saccharomyces* VIN13. Results by Benito et al. (2015) pointed out that fermentations with *K. thermotolerans* produced higher levels of total terpenes, α-terpineol, and specially nerol while *P. kluyveri* produced the highest reported levels in hotrienol and linalool oxide. Particularly interesting non-*Saccharomyces* yeast in this area is *T. delbrueckii* as it has together with *Kluyveromyces lactis* a high β-glucosidase activity and high ability to biotransform monoterpene alcohols translocating geraniol and nerol to linalool or reducing geraniol to citronellol (King and Richard Dickinson, 2000). A study by Garcia et al. (2002) showed the positive influence of mixed cultures of *D. vanriji* and *S. cerevisiae* on the concentration of geraniol, due to the high activity of β-glucosidase showed by this non-*Saccharomyces* strain. According to Cus and Jenko (2013) use of selected hybrid of *S. cerevisiae* and *Saccharomyces paradoxus* alone or with a combination of *Saccharomyces* and *T. delbrueckii* strains influenced free monoterpene alcohols concentrations what resulted with the better flavor and overall quality of Traminer wine compared to reference *S. cerevisiae* strain, even though the total concentration of free volatile monoterpenes produced by *T. delbrueckii* and selected hybrid were lower compared to *S. cerevisiae* strain. Monoterpene composition of Vino santo wines produced using multistarter *T. delbrueckii*, and *S. cerevisiae* strain showed differences, primarily in the higher concentrations of endiol, linalool, and α-terpineol compared to wines produced with spontaneous fermentation and *S. cerevisiae* single strain (Azzolini et al., 2015). Marked influence of *T. delbrueckii* in simultaneously and sequentially fermentation with *S. cerevisiae* compared to control (*S. cerevisiae*) fermentation on the aroma of Amarone wine was noted by Azzolini et al. (2012). Their results presented higher linalool, hotrienol, α-terpineol, endiol as well as total terpenes concentrations in wines made with mixed *T. delbrueckii*+*S. cerevisiae* wines. Sequential inoculation of *T. delbrueckii* and *S. cerevisiae* significantly contributed to the quality of Chardonnay and Palomino wine even though for terpenes concentration no significant differences were noted between control and inoculated fermentations (Puertas et al., 2017). The oenological utilization of *H. uvarum* in simultaneous and sequential cofermentation with *S. cerevisiae* pointed out higher concentrations of α-terpineol as well as the higher production of some other classes of volatiles terpenes, esters, acids, and alcohols (Tristezza et al., 2016). Inoculation of Macabeo must with either *Hanseniaspora vineae* or *S. cerevisiae* yeast resulted with some differences in terpenes composition being higher in *H. vineae* wines even though the concentrations of these compounds (citronellol, α-terpineol) were under their odour threshold, so without any contribution to the wine flavor profile (Lleixà et al., 2016). In the work by Englezos et al. (2016) *S. bacillaris* (synonym *C. zemplinina*) and *S. cerevisiae* were evaluated in mixed (coinoculated and sequential) fermentations with the aim of improving the aroma profile of Barbera wine. The production of free terpenes was found to be much higher in the mixed fermentations compared to the pure

S. cerevisiae fermentations what was in agreement with already reported data by Sadoudi et al. (2012) and Whitener et al. (2016). *M. pulcherrima* has long been associated with grapes and wine and early research into the potential of this strain showed that certain isolates displayed a high β-glucosidase activity (Fernández et al., 2000; González-Pombo et al., 2008). Unfortunately, in the study by Benito et al. (2015) *M. pulcherrima* in sequential fermentation with *S. cerevisiae* did not produce significant concentrations of any volatile terpene compound (Table 6.2).

6.2.3 Yeast Influence on C_{13}-Norisoprenoids Composition

In grape berry, C_{13}-norisoprenoids are degradations products of carotenoids. The most amount of these compounds is contained in nonvolatile precursors. During the vinification, these nonvolatile compounds are cleavage by the action of β-glycosidases and release of aglycons occur. In general, C_{13}-norisoprenoids can be divided into megastigmanes and nonmegastigmanes. The most abundant megastigman in wine is β-damascenone, followed by β-ionone and α-ionone. Nonmegastigmanes norisoprenoids can be very active aroma compounds in wine. The most important are 1,1,6-trimethyl-1,2-dihydronapthalene (TDN), (*E*)-1-(2,3,6-trimethylphenyl)-buta-1, 3-diene (TPB), and vitispiranes (Loscos et al., 2007; Hernandez-Orte et al., 2008; Skouroumounis and Sefton, 2001).

Sequential inoculation by commercial *P. kluyveri* and *M. pulcherrima* yeasts with *S. cerevisiae* compared to pure *S. cerevisiae* and sequential fermentation with *L. thermotolerans* commercial yeast showed significant influence on the concentrations of β damascenone, but no impact was noted on the concentrations of vitispirane (Benito et al., 2015). Use of *T. delbrueckii* in sequential fermentation with *S. cerevisiae* and compared to single-culture *S. cerevisiae* fermentation also did not show any significant difference in β damascenone (Azzolini et al., 2015). On the contrary, the work by Englezos et al. (2016) showed the positive influence of *S. bacillaris* as a single culture as well as in simultaneous and sequential fermentation with *S. cerevisiae* on the concentrations of β damascenone was noted. In the study by Sadoudi et al. (2012), comparison of *S. cerevisiae* and three non-*Saccharomyces* yeasts (*C. zemplinina*, *T. delbrueckii*, and *M. pulcherrima*) were used to conduct fermentations either in monoculture or coculture. *C. zemplinina* as a single culture and *M. pulcherrima* in coculture were characterized by the higher production of norisoprenoides, especially with β damascenone. The difference in norsoprenoide production was also noted by Whitener et al. (2015) who studied early fermentation characteristics of *Kazachstania gamospora, L. thermotolerans, M. pulcherrima, T. delbrueckii*, and *Zygosaccharomyces kombuchaensis* and *S. cerevisiae*

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Table 6.2 Dependence of Monoterpenes Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast

Yeast	Linalool	α-Terpineol	Citronellol	Linalool Oxide 1	Nerol Oxide	Linalool Oxide 2	Hotrineol	Nerol	Geraniol	Refs.
S. cerevisiae	0.02	na	na	na	na	na	na	na	na	Englezos et al. (2016)
Starm. bacillaris	0.02	na	na	na	na	na	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* coinoculation	0.02	na	na	na	na	na	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* sequential	0.02	na	na	na	na	na	na	na	na	Englezos et al. (2016)
H. vineae	12	112	27	na	na	na	na	na	na	Lleixà et al. (2016)
S. cerevisiae	28	na	39	na	na	na	na	na	na	Lleixà et al. (2016)
H. uvarum + *S. cerevisiae* simultaneous	na	73	na	na	na	na	na	na	na	Tristezza et al. (2016)
H. uvarum + *S. cerevisiae* sequential	na	50	na	na	na	na	na	na	na	Tristezza et al. (2016)
S. cerevisiae		30	na	na	na	na	na	na	na	Tristezza et al. (2016)
S. bayanus	21.6	14.84	na	9.28	22.66	3.79	47.65	220.11	4	Benito et al. (2015)
K. thermotolerans + *S. bayanus*	21.9	16.21	na	10.15	21.38	3.8	57.35	267.87	3	Benito et al. (2015)
P. kluyveri + *S. bayanus*	21.21	14.08	na	20.56	22.24	3.72	68.38	161.93	na	Benito et al. (2015)
M. Pulcherrima + *S. bayanus*	21.58	14.07	na	10.08	21.91	3.76	57.25	162.15	1	Benito et al. (2015)

Continued

Table 6.2 Dependence of Monoterpenes Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Linalool	α-Terpineol	Citronellol	Linalool Oxide 1	Nerol Oxide	Linalool Oxide 2	Hotrineol	Nerol	Geraniol	Refs.
S. cerevisiae	4.4	1.9	3.1	na	na	na	na	1.5	3.9	Azzolini et al. (2015)
T. delbruecki1 + *S. cerevisiae*	4.0	2.5	3.2	na	na	na	na	2.9	5.6	Azzolini et al. (2015)
T. delbruecki2 + *S. cerevisiae*	3.6	2.1	2.7	na	na	na	na	1.4	4.4	Azzolini et al. (2015)
S. cerevisiae	9.5	5.4	2.1	na	na	na	na	1.9	3.6	Azzolini et al. (2015)
T. delbruecki1 + *S. cerevisiae*	10.8	7.2	4.5	na	na	na	na	1.4	3.8	Azzolini et al. (2015)
T. delbruecki 2+ *S. cerevisiae*	10.5	5.5	1.6	na	na	na	na	2.4	4.6	Azzolini et al. (2015)
S. cerevisiae	34.4	18.6	112.9	na	na	na	9.6	20.5	104.1	Furdíková et al. (2014)
S. cerevisiae	40	21.2	128.6	na	na	na	6.8	17.4	105.0	Furdíková et al. (2014)
S. cerevisiae	28.1	15.3	95.6	na	na	na	6.4	17.3	113.9	Furdíková et al. (2014)
S. cerevisiae	27.3	16.0	101.1	na	na	na	na	23.1	129.8	Furdíková et al. (2014)
S. cerevisiae	41	57	54	na	na	na	na	72	726	Cus and Jenko (2013)
S. cerevisiae	38	80	78	na	na	na	na	42	433	Cus and Jenko (2013)
T. delbruecki + *S. cerevisiae*	49	109	57	na	na	na	na	40	488	Cus and Jenko (2013)
S. cerevisiae	33.98	na	34.75	na	na	na	na	na	27.77	Sadoudi et al. (2012)
M. pulcherrima	30.82	na	–	na	na	na	na	na	66.87	Sadoudi et al. (2012)
T. delbrueckii	27.38	na	29.91	na	na	na	na	na	40.99	Sadoudi et al. (2012)
T. delbruecki + *S. cerevisiae*	49	109	57	na	na	na	na	40	488	Cus and Jenko (2013)
S. cerevisiae	33.98	na	34.75	na	na	na	na	na	27.77	Sadoudi et al. (2012)
M. pulcherrima	30.82	na	–	na	na	na	na	na	66.87	Sadoudi et al. (2012)
T. delbrueckii	27.38	na	29.91	na	na	na	na	na	40.99	Sadoudi et al. (2012)

C. zemplinina	68.88	na	16.16	na	na	na	22.56	na	159.8	Sadoudi et al. (2012)
C. zemplinina + *S. cerevisiae*	41.46	na	60.45	na	na	na	na	na	99.23	Sadoudi et al. (2012)
M. pulcherrima + *S. cerevisiae*	51.42	na	21.13	na	na	na	51.90	na	30.97	Sadoudi et al. (2012)
T. delbruecki + *S. cerevisiae*	44.48	na	77.72	na	na	na	9.56	na	74.84	Sadoudi et al. (2012)
C. pulcherrima	162.8	58.6	na	na	na	na	na	13.7	36.3	Rodriguez et al. (2010)
S. cerevisiae	215.9	22.7	na	na	na	na	na	3.7	10.3	Rodriguez et al. (2010)
C. pulcherrima + *S. cerevisiae* simulaneous	200.5	24.5	na	na	na	na	na	3.2	9.9	Rodriguez et al. (2010)
C. pulcherrima + *S. cerevisiae* sequential	234.4	32.4	na	na	na	na	na	4.6	11.3	Rodriguez et al. (2010)
D. pseudopolymorphus	34.7	36.4	3.1	na	na	na	na	4.6	7.4	Cordero Otero et al. (2003)
S. cerevisiae	34.9	35.7	7.2	na	na	na	na	5.6	1.3	Cordero Otero et al. (2003)
D. pseudopolymorphus + *S. cerevisiae*	33.6	33.3	37.2	na	na	na	na	7.9	21.2	Cordero Otero et al. (2003)
S. cerevisiae	36.8	74.8	na	132.1	na	34.3	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	36.3	74.8	na	134.5	na	34.3	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	41.3	74.0	na	132.6	na	33.0	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	42.6	74.7	na	133.1	na	32.2	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	41.4	74.2	na	130.6	na	32.6	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	42.9	76.9	na	127.0	na	31.8	na	na	na	Srisamatthakarn (2011)

Continued

Table 6.2 Dependence of Monoterpenes Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Linalool	α-Terpineol	Citronellol	Linalool Oxide 1	Nerol Oxide	Linalool Oxide 2	Hotrineol	Nerol	Geraniol	Refs.
Saccharomyces spp.	40.0	74.0	na	129.4	na	32.3	na	na	na	Srisamatthakarn (2011)
Saccharomyces spp.	42.6	76.8	na	134.0	na	32.9	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	40.6	78.5	na	134.6	na	32.9	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	38.2	76.5	na	134.8	na	33.8	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	40.0	79.3	na	136.8	na	33.6	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	40.3	73.6	na	127.9	na	32.1	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	39.2	70.4	na	129.0	na	32.2	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	511.6	77.4	83.1	na	na	na	na	na	99.6	Garcia et al. (2002)
D. vanriji + *S. cerevisiae*	526.7	68.8	63.3	na	na	na	na	na	141.2	Garcia et al. (2002)
D. vanriji + *S. cerevisiae*	458.2	67.1	61.5	na	na	na	na	na	118.9	Garcia et al. (2002)

na, not analyzed.
Results are expressed in μg/L.

used as a control in both Sauvignon blanc and Syrah musts. Between them, *L. thermotolerans* showed no ability to produce β-damascenone but was among the highest producers of vitispirane and TDN. *S. cerevisiae* was a good producer of all norisoprenoids analyzed while *K. gamospora* produced the highest quantities of β damascenone (Table 6.3).

6.3 Secondary (Fermentation) Aroma of Wine

The composition and sensory character of the wine are dependent on the dominant yeasts and their metabolism during fermentation as well as by the grape variety and viticultural practices. Secondary aromas consisting of volatile fatty acids, higher alcohols, acetate, and ethyl esters are mainly produced throughout fermentation process, and biosynthesis of these compounds is species and strain dependent. This method combines biochemical assimilation and dissimilation of nutrients by yeast cells and is mediated by dehydrogenase enzymes (Lambrechts and Pretorius, 2000). The synthesis of these compounds have been studied mostly in *S. cerevisiae*, but nowadays research has also expanded to other non-*Saccharomyces* yeasts (Benito et al., 2016a; Ciani et al., 2006; Dashko et al., 2015; Sadoudi et al., 2012; Zott et al., 2011). Use of selected yeast strain can ensure a reproducible product, reduce the risk of wine spoilage, and allow a more predictable control of fermentation and quality (Romano et al., 2003). So for the producers, it is important to know the potential differences in volatile fermentation compounds synthesis by various yeast strains to select the best one that can positively improve final wine quality. Usually, for the production of young white wines high producers of acetate and ethyl esters and low producers of higher alcohols yeast strains are selected with the aim of increasing desirable fruity aromas and taste.

6.3.1 Yeast Influence on Volatile Fatty Acids

Different straight-chain fatty acids are contained in wine. Base on the number of C-atoms they can be divided on short-chain (C_2-C_4), medium-chain (C_5-C_{10}), long-chain (C_{10}-C_{18}), and branched-chain (2-methyl propanoic, 2-methyl butanoic, and 3-methyl butanoic acid) fatty acids. Medium straight-chain fatty acids are products of saturated fatty acid metabolism. Acetyl-CoA carboxylase catalyzed the formation of malonyl-CoA from acetyl-CoA. The synthase enzyme complex increases chain length by two C-units (Bardi et al., 1998). Medium-chain fatty acid (butyric, hexanoic, octanoic, decanoic) are produced primarily by yeast, having an essential role in the biosynthesis of long-chain fatty acids as well as being one of the main substrates in

Table 6.3 Dependence of C_{13}-Norisoprenoids Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast

Yeast	β-Damascenone	Vitispirane	Actinidol 1	Actinidol 2	1,1,6-Trimethyl-1, 2-dihydronaphthalene (TDN)	Refs.
S. cerevisiae	3	na	na	na	na	Englezos et al. (2016)
Starm. bacillaris	6	na	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* coinoculation	8	na	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* sequential	8	na	na	na	na	Englezos et al. (2016)
S. bayanus	na	13.19	na	na	na	Benito et al. (2015)
K. thermotolerans + *S. bayanus*	na	13.31	na	na	na	Benito et al. (2015)
P. kluyveri + *S. bayanus*	na	14.24	na	na	na	Benito et al. (2015)
M. Pulcherrima + *S. bayanus*	na	15.21	na	na	na	Benito et al. (2015)
S. cerevisiae	6.6	na	1.5	3.8	na	Azzolini et al. (2015)
T. delbruecki1 + *S. cerevisiae*	4.7	na	1.1	2.6	na	Azzolini et al. (2015)
T. delbruecki2 + *S. cerevisiae*	6.9	na	1	2.4	na	Azzolini et al. (2015)
S. cerevisiae	6.1	na	2.3	6	na	Azzolini et al. (2015)
T. delbruecki1 + *S. cerevisiae*	6.0	na	2.9	6.2	na	Azzolini et al. (2015)
T. delbruecki2 + *S. cerevisiae*	5.9	na	2.1	5.2	na	Azzolini et al. (2015)
S. cerevisiae	36.44	na	na	na	na	Sadoudi et al. (2012)
M. pulcherrima	27.21	na	na	na	na	Sadoudi et al. (2012)
T. delbrueckii	29.66	na	na	na	na	Sadoudi et al. (2012)
C. zemplinina	64.55	na	na	na	na	Sadoudi et al. (2012)
C. zemplinina + *S. cerevisiae*	30.25	na	na	na	na	Sadoudi et al. (2012)
M. pulcherrima + *S. cerevisiae*	65.82	na	na	na	na	Sadoudi et al. (2012)
T. delbruecki + *S. cerevisiae*	31.99	na	na	na	na	Sadoudi et al. (2012)
S. cerevisiae	9.5	2.2	na	na	0.36	Whitener et al. (2015)
S. cerevisiae	7.1	1.0	na	na	0.18	Whitener et al. (2015)
K. gamospora	27.2	0.48	na	na	na	Whitener et al. (2015)

K. gamospora	na	0.53	na	na	0.24	Whitener et al. (2015)
L. thermotolerans	na	1.7	na	na	0.23	Whitener et al. (2015)
L. thermotolerans	na	1.1	na	na	0.13	Whitener et al. (2015)
M. pulcherrima	17.2	0.42	na	na	na	Whitener et al. (2015)
M. pulcherrima	na	0.50	na	na	na	Whitener et al. (2015)
T. delbruckii	18.3	0.45	na	na	na	Whitener et al. (2015)
T. delbruckii	na	0.45	na	na	na	Whitener et al. (2015)
Z. kombuchaensis	14.05	0.36	na	na	0.24	Whitener et al. (2015)
Z. kombuchaensis	5.1	0.25	na	na	0.11	Whitener et al. (2015)

na, not analyzed.
Results are expressed in μg/L.

ethyl esters formation. Their higher content in wine can have negative impact emphasizing greasy, rancid, and cheesy odors.

According to Liu et al. (2017), single *S. cerevisiae* produced the highest levels of 3-methylbutanoic acid, hexanoic acid, and octanoic acid compared with the wines made with sequential inoculation of *H. uvarum* and *S. cerevisiae.* On the contrary in the work by Tristezza et al. (2016) mixed fermentations of *H. uvarum* and *S. cerevisiae* resulted in decreases in isovaleric acid and increases in hexanoic, octanoic acid, and decanoic acid. Fermentation with two indigenous *S. cerevisiae* and spontaneous microflora had no marked effect on the concentration of main fatty acids even though a bit higher concentrations of octanoic acid were noticed in spontaneous fermentation wines (Cordero-Bueso et al., 2016). Results of pure *H. vineae* and *S. cerevisiae* fermentation demonstrated that *S. cerevisiae* produced higher concentrations of main fatty acids except for isobutyric acid, one that was significantly present in *H. vineae* wines (Lleixà et al., 2016). The mixed culture of *C. stellata* with *S. cerevisiae* produced the highest amounts of medium-chain fatty acids (hexanoic acid`` and octanoic acid), followed by *Pichia fermentas* and *M. pulcherrima* (Liu et al., 2016). These results are in accordance with Andorrà et al. (2012) who found out the notable increase of fatty acids, especially butyric acid, by non-*Saccharomyces* species (*C. zemplinina*, *H. uvarum*) in single as well as in mixed fermentations. Andorrà et al. (2010) pointed out a higher synthesis of only isobutyric acid by *C. zemplinina* compared to *S. cerevisiae* while on the other hand concentrations of main medium-chain fatty acids were always higher in the *S. cerevisiae* fermentations compared with non-*Saccharomyces* yeasts. The use of mixed-culture fermentation with *S. bacillaris* (synonym *C. zemplinina*) and *S. cerevisiae* showed no significant differences in fatty acids production, with the only exception of the dodecanoic acid, which was produced in higher quantities in the coinoculated trials (Englezos et al., 2016). Higher concentration of decanoic acid was found by Comitini et al. (2011) in the fermentation carried out by mixed culture of *C. zemplinina* and *S. cerevisiae*. No significant difference in the hexanoic and octanoic acid concentrations was determined in the combined fermentations with *L. thermotolerans* and *S. cerevisiae* compared to a single fermentation by *S. cerevisiae* (Benito et al., 2016a). Three yeast strains (*Kluyveromyces marxianus*, *S. cerevisiae*, *T. delbrueckii*) were selected and separately inoculated in vinifications experiments at a Georgian cellar. As regards the short- and medium-chain fatty acids yeast-related differences were modest and concerned only the hexanoic acid being higher in the wines obtained with *S. cerevisiae* and isovaleric acid whose concentration was higher in the wine produced by non-*Saccharomyces* yeasts. Chemical analysis of Soave and Chardonnay dry wines showed that multistarter fermentation with *T. delbrueckii* and *S. cerevisiae* yeasts greatly affected the content of C_4-C_{10} fatty acids. The amount of the total fatty acid was two to four times lower than in control *S. cerevisiae* wines. Greater differences were observed for hexanoic and octanoic acid, the most abundant fatty acids and also decanoic acid whose concentration decreased under its threshold (Azzolini et al., 2015). Sequential inoculation with *T. delbrueckii* or *M. pulcherrima* and *S. cerevisiae* in base wine production had no marked influence on the concentration of fatty acids except hexanoic acid whose concentration was higher in wine produced with *S. cerevisiae* (González-Royo et al., 2015). Multistarter fermentation trial carried out by inoculating non-*Saccharomyces* yeasts (*M. pulcherrima*. *L. thermotolerans*, *T. delbrueckii*, *C. zemplinina*) in combination with the *S. cerevisiae* strain as a starter showed positive influence of *M. pulcherrima* on medium-chain fatty acids and lower production of octanoic acid by *L. thermotolerans* and *C. zemplinina* compared to pure *S. cerevisiae* (Comitini et al., 2011). The effect of sequential inoculation of *Wickerhamomyces anomalus* (formerly *Hanseniaspora anomala*) and a commercial *S. cerevisiae* strain in red musts from Mazuela variety has been examined by Cañas et al. (2014). The wines made by sequential fermentation presented lower concentrations of octanoic and hexanoic acid. The data by Lombard (2016) demonstrated that sequential culture fermentations with *W. anomalus* and a commercial *S. cerevisiae* strain increased fatty acid concentrations of butyric, hexanoic, and octanoic acid and in contrast, hexanoic and octanoic acid amount was decreased in sequential culture fermentations with *Kazachstania aerobia*. *K. aerobia* is not a high producer of fatty acids, as similarly observed with the non-*Saccharomyces* yeast *T. delbrueckii*, suggesting that some non-*Saccharomyces* yeasts metabolism does not result in excessive fatty acid biosynthesis (Azzolini et al., 2015). Regarding the production of fatty acids, *Hanseniaspora guilliermondii* was, according to Lage et al. (2014) a high producer when grown in single culture, primarily due to the amount of isobutyric acid produced. On the contrary, *S. cerevisiae* fermented wines had more isovaleric acid, while total concentration of fatty acids was more or less equal in wines fermented by single *S. cerevisiae* or *H. guilliermondii* and *S. cerevisiae* mixed-culture. A study by Moreira et al. (2011) showed that *H. guilliermondii* inoculated fermentation led to the obtaining of wine with lower contents in pentanoic, hexanoic, and octanoic acids than the wine produced from spontaneous fermentation. Medium-chain fatty acid concentrations also decreased in wines obtained from mixed cultures of *Hanseniaspora osmophila* and *S. cerevisiae*. Reductions of about 30%–40% in the amounts of hexanoic, octanoic, and decanoic acids were observed, in relation to *S. cerevisiae* monoculture fermentations (Viana et al., 2009; Table 6.4).

Table 6.4 Dependence of Fatty Acids Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast

Yeast	Pentanoic Acid	Hexanoic Acid	Octanoic Acid	Decanoic Acid	3-Methylbutanoic Acid	Isobutyric Acid	Butyric Acid	Refs.
S. cerevisiae	1.65	7.0	4.84	0.68	na	na	na	Belda et al. (2017)
S. cerevisiae	1.63	7.7	5.86	1.10	na	na	na	Belda et al. (2017)
T. delbrueckii + *S. cerevisiae*	1.49	7.4	5.15	1.45	na	na	na	Belda et al. (2017)
T. delbrueckii + *S. cerevisiae*	1.45	7.5	5.38	1.39	na	na	na	Belda et al. (2017)
S. cerevisiae	na	1.8	1.7	0.4	1.2	na	na	Vigentini et al. (2016)
T. delbrueckii	na	1.5	1.3	0.3	3.5	na	na	Vigentini et al. (2016)
K. marxianus	na	1.5	1.5	0.3	3.8	na	na	Vigentini et al. (2016)
S. cerevisiae	na	8.24	4.32	na	na	na	na	Benito et al. (2016a)
L. thermotolerans + *S. cerevisiae*	na	8.32	4.44	na	na	na	na	Benito et al. (2016a)
L. thermotolerans + *S cerevisiae*	na	8.18	3.16	na	na	na	na	Benito et al. (2016a)
H. vineae	na	0.33	0.734	0.979	na	0.074	na	Lleixà et al. (2016)
S. cerevisiae	na	0.777	1.757	0.389	na	na	na	Lleixà et al. (2016)
H. uvarum+ *S. cerevisiae*	na	2.366	4.716	1.178	0.244	0.166	0.115	Tristezza et al. (2016)
H. uvarum+ *S. cerevisiae*	na	2161	4372	1344	269	93	83	Tristezza et al. (2016)
S. cerevisiae	na	2159	3922	1278	434	212	85	Tristezza et al. (2016)
S. cerevisiae	na	0.85	1.51	na	2.01	na	0.10	Varela et al. (2016)
M. pulcherrima + *S. cerevisiae*	na	1.10	1.26	na	3.60	na	0.07	Varela et al. (2016)
S. uvarum + *S. cerevisiae*	na	0.36	0.31	na	1.61	na	1.54	Varela et al. (2016)

M. pulcherrima + *S. uvarum* + *S. cerevisiae*	na	0.29	0.30	na	1.70	na	1.48	Varela et al. (2016)
S. cerevisiae	na	1.14	1.09	na	1.29	na	0.08	Varela et al. (2016)
M. pulcherrima + *S. cerevisiae*	na	1.44	1.16	na	1.34	na	0.38	Varela et al. (2016)
S. uvarum + *S. cerevisiae*	na	0.26	0.29	na	1.10	na	1.67	Varela et al. (2016)
M .pulcherrima + *S. uvarum* + *S. cerevisiae*	na	1.80	0.50	na	0.80	na	1.74	Varela et al. (2016)
S. cerevisiae	na	4.62	20.11	0.6	1.04	na	1.27	González-Royo et al. (2015)
T. delbrueckii + *S. cerevisiae*	na	3.81	16.34	0.3	0.98	na	1.03	González-Royo et al. (2015)
M. pulcherrima + *S. cerevisiae*	na	3.75	15.86	0.3	1.07	na	1.04	González-Royo et al. (2015)
S. bayanus	na	11.98	6.72	3.1	na	na	na	Benito et al. (2015)
K. thermotolerans + *S. bayanus*	na	11.5	5.92	3.39	na	na	na	Benito et al. (2015)
P. kluyveri+ *S. bayanus*	na	12.58	6.91	3.19	na	na	na	Benito et al. (2015)
M. Pulcherrima + *S. bayanus*	na	12.82	6.55	2.92	na	na	na	Benito et al. (2015)
S. cerevisiae	0.018	1.28	1.31	0.25	na	0.26	0.18	Cañas et al. (2014)
W. anomalus + *S. cervisiae*[a]	0.015	0.99	1.08	0.22	na	0.23	0.14	Cañas et al. (2014)
S. cerevisiae	na	1.958	4.328	1.200	na	na	0.336	Azzolini et al. (2015)
T. delbrueckii1 + *S. cerevisiae*	na	0.779	1.089	0.342	na	na	0.361	Azzolini et al. (2015)
T. delbrueckii2 + *S. cerevisiae*	na	0.789	1.221	1.151	na	na	0.297	Azzolini et al. (2015)
S. cerevisiae	na	1.792	3.707	1.180	na	na	0.319	Azzolini et al. (2015)

Continued

Table 6.4 Dependence of Fatty Acids Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Pentanoic Acid	Hexanoic Acid	Octanoic Acid	Decanoic Acid	3-Methylbutanoic Acid	Isobutyric Acid	Butyric Acid	Refs.
T. delbruecki1 + *S. cerevisiae*	na	0.651	0.500	0.182	na	na	0.268	Azzolini et al. (2015)
T. delbruecki2 + *S. cerevisiae*	na	0.648	0.503	0.596	na	na	0.255	Azzolini et al. (2015)
S. cerevisiae	na	1.26	1.53	na	na	2.67	1.17	Lage et al. (2014)
H. guilliermondii + *S. cerevisiae*	na	1.43	1.92	na	na	3.33	1.25	Lage et al. (2014)
H. guilliermondii	na	0.19	0.41	na	na	12.74	0.33	Lage et al. (2014)
S. cerevisiae	na	na	0.06	2.79	na	na	na	Furdíková et al. (2014)
S. cerevisiae	na	na	0.06	3.41	na	na	na	Furdíková et al. (2014)
S. cerevisiae	na	na	0.12	4.95	na	na	na	Furdíková et al. (2014)
S. cerevisiae	na	na	0.02	1.24	na	na	na	Furdíková et al. (2014)
H. vineae + *S. cerevisiae*	na	1902	4184	4607	187	512	366	Medina et al. (2013)
S. cerevisiae- spontaneous	na	3.990	0.010	4.713	0.664	0.590	0.439	Medina et al. (2013)
S. cerevisiae	na	3.834	8.478	3.796	0.748	0.305	0.431	Medina et al. (2013)
S. cerevisiae	na	2.7	4.3	1.2	0.6	4.0	0.6	Contreras et al. (2014)
M. pulcherrima + *S. cerevisiae*	na	1.9	3.0	0.8	0.1	2.3	0.7	Contreras et al. (2014)
S. cerevisiae	na	2.4	1.7	0.4	1.7	4.6	1.1	Contreras et al. (2014)
M. pulcherrima + *S. cerevisiae*	na	1.9	1.0	0.3	1.5	3.7	1.0	Contreras et al. (2014)
S. cerevisiae	na	na	0.586	0.038	na	na	na	Sadoudi et al. (2012)
M. pulcherrima	na	na	0.079	0.003	na	na	na	Sadoudi et al. (2012)
T. delbrueckii	na	na	0.369	0.005	na	na	na	Sadoudi et al. (2012)

C. zemplinina	na	na	na	0.026	na	na	na	Sadoudi et al. (2012)
C. zemplinina + *S. cerevisiae*	na	na	0.470	0.246	na	na	na	Sadoudi et al. (2012)
M. pulcherrima + *S. cerevisiae*	na	na	1.305	0.286	na	na	na	Sadoudi et al. (2012)
T. delbruecki + *S. cerevisiae*	na	na	0.269	0.137	na	na	na	Sadoudi et al. (2012)
S. cerevisiae	1.07	0.58	2.15	0.53	na	2.51	1.64	Moreira et al. (2011)
H. guilliermondii NCYC 2380	0.85	0.34	1.40	0.21	na	2.80	1.18	Moreira et al. (2011)
S. cerevisiae	na	3.42	2.62	1.59	na	1.77	0.64	Andorrà et al. (2010)
C. zemplina	na	0.31	0.23	0.12	na	29.08	0.34	Andorrà et al. (2010)
C. zempina + *S. cerevisiae*	na	2.89	1.88	0.84	na	12.66	0.75	Andorrà et al. (2010)
H. uvarum + *S. cerevisiae*	na	1.83	0.92	0.69	na	6.13	0.77	Andorrà et al. (2010)
C. zemplina + *H. uvarum* + *S. cerevisiae*	na	1.74	1.62	0.56	na	4.35	0.64	Andorrà et al. (2010)
T. delbrueckii	na	0.12	0.09	0.22	na	1.55	0.17	Renault et al. (2009)
S. cerevisiae	na	4.377	7.447	3.197	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	5.575	8.474	3.155	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	3.921	6.831	3.802	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	6.040	10.495	4.860	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	6.468	9.457	3.991	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	5.289	8.315	3.982	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	4.207	6.870	3.381	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	2.3	3.0	0.51	na	na	na	Viana et al. (2008)
H. guilliermondii	na	0.58	0.42	0.54	na	na	na	Viana et al. (2008)
H. guilliermondii	na	0.51	0.26	0.62	na	na	na	Viana et al. (2008)
H. osmophila	na	0.76	0.15	0.55	na	na	na	Viana et al. (2008)
P. membranifaciens	na	0.52	0.53	0.45	na	na	na	Viana et al. (2008)
S. cerevisiae	na	0.15	0.04	0.01	na	0.036	na	Gil et al. (1996)

Continued

Table 6.4 Dependence of Fatty Acids Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Pentanoic Acid	Hexanoic Acid	Octanoic Acid	Decanoic Acid	3-Methylbutanoic Acid	Isobutyric Acid	Butyric Acid	Refs.
S. cerevisiae	na	0.20	0.04	–	na	0.01	na	Gil et al. (1996)
S. bayanus	na	0.19	0.05	0.03	na	0.012	na	Gil et al. (1996)
H. uvarum	na	–	0.02	0.02	na	0.026	na	Gil et al. (1996)
K. apiculata	na	–	0.02	0.01	na	0.011	na	Gil et al. (1996)
S. cerevisiae	na	0.20	0.58	0.52	na	na	na	Englezos et al. (2016)
Starm. bacillaris	na	0.19	0.49	0.38	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* coinoculation	na	0.25	0.66	0.73	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* sequential	na	0.24	0.52	0.49	na	na	na	Englezos et al. (2016)
S. cerevisiae	na	0.929	1.207	0.138	na	na	na	Hu et al. (2018)
H. uvarum + *S. cerevisiae*	na	1.279	2.082	0.331	na	na	na	Hu et al. (2018)
H. uvarum + *S. cerevisiae*	na	0.746	0.885	0.141	na	na	na	Hu et al. (2018)
S. cerevisiae	na	4.97	6.11	3.70	0.08	na	1.90	Cordero-Bueso et al. (2013)
C. stellata	na	0.34	0.42	0.12	0.31	na	0.49	Cordero-Bueso et al. (2013)
H. guilliermondii	na	1.23	0.50	0.71	0.08	na	0.44	Cordero-Bueso et al. (2013)
L. fermentati	na	0.94	0.36	0.52	0.14	na	0.27	Cordero-Bueso et al. (2013)

L. thermotolerans	na	0.21	0.20	0.12	0.59	na	0.81	Cordero-Bueso et al. (2013)
P. kudriavzevii	na	0.24	0.27	0.19	0.39	na	0.45	Cordero-Bueso et al. (2013)
P. membranifaciens	na	0.67	0.20	0.06	0.17	na	0.21	Cordero-Bueso et al. (2013)
S. pombe	na	1.99	0.80	0.44	0.19	na	0.51	Cordero-Bueso et al. (2013)
T. delbrueckii	na	2.31	9.88	2.88	0.22	na	0.54	Cordero-Bueso et al. (2013)
W. anomalus	na	0.31	0.59	0.21	0.13	na	0.45	Cordero-Bueso et al. (2013)
S. cerevisiae Vin7-*S. cerevisiae/ S. kudriavzev-*	na	7.6	8.5	3.7	0.75	1.3	1.5	King et al. (2008)
S. cerevisiae	na	7.6	10	4.0	0.46	0.65	1.3	King et al. (2008)
S. cerevisiae	na	6.6	10	5.0	0.55	1.0	1.5	King et al. (2008)
S. cerevisiae Vin7/ QA23-coinoculation	na	6.2	9.8	3.9	0.49	0.98	1.4	King et al. (2008)
S. cerevisiae Vin7/ Vin13-coinoculation	na	6.4	8.7	4.5	0.56	1.0	1.6	King et al. (2008)
S. cerevisiae spontaneous	na	4.97	6.11	3.70	0.08	3.01	1.90	Cordero-Bueso et al. (2016)
S. cerevisiae	na	4.29	5.45	3.68	0.26	3.02	1.93	Cordero-Bueso et al. (2016)
S. cerevisiae	na	4.54	5.07	2.85	0.02	2.91	1.31	Cordero-Bueso et al. (2016)
S. cerevisiae	na	9.0	9.3	2.0	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	12.7	11.0	1.6	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	7.0	7.0	1.6	na	na	na	Srisamatthakarn (2011)

Continued

Table 6.4 Dependence of Fatty Acids Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Pentanoic Acid	Hexanoic Acid	Octanoic Acid	Decanoic Acid	3-Methylbutanoic Acid	Isobutyric Acid	Butyric Acid	Refs.
S. cerevisiae	na	7.0	7.3	2.0	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	10.0	8.7	1.9	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	9.7	10.0	2.5	na	na	na	Srisamatthakarn (2011)
Saccharomyces spp.	na	12.3	11.0	2.6	na	na	na	Srisamatthakarn (2011)
Saccharomyces spp.	na	13.0	12.0	2.7	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	13.3	12.7	2.9	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	13.0	12.3	2.7	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	14.0	13.0	2.8	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	8.3	9.0	2.0	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	10.0	9.0	1.9	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	5.72	na	1.66	na	0.91	11.57	4.13	Andorrà et al. (2012)
C. zemplinina	1.37	na	–	na	–	9.42	46.82	Andorrà et al. (2012)
C. zemplinina + *S. cerevisiae*	5.05	na	1.24	na	2.10	12.88	33.88	Andorrà et al. (2012)
H. uvarum + *S. cerevisiae*	7.66	na	1.25	na	2.56	17.30	28.29	Andorrà et al. (2012)

C. zemplinina + *H. uvarum* + *S. cerevisiae*	8.74	na	–	na	1.35	19.68	29.47	Andorrà et al. (2012)
W. anomalus	na	2.74	2.77	na	na	na	1.20	Lombard (2016)
W. anomalus	na	2.82	2.83	na	na	na	1.23	Lombard (2016)
K. aerobia	na	1.25	1.55	na	na	na	0.67	Lombard (2016)
K. aerobia	na	1.51	1.93	na	na	na	0.71	Lombard (2016)
W. anomalus + *S. cerevisiae*	na	3.69	3.74	na	na	na	1.65	Lombard (2016)
W. anomalus + *S. cerevisiae*	na	3.34	3.37	na	na	na	1.58	Lombard (2016)
K. aerobia + *S. cerevisiae*	na	2.29	2.47	na	na	na	1.10	Lombard (2016)
K. aerobia + *S. cerevisiae*	na	2.42	2.57	na	na	na	1.23	Lombard (2016)
S. cerevisiae	na	3.25	3.29	na	na	na	1.42	Lombard (2016)

Results are expressed in mg/L.

[a] *na*, not analyzed.

6.3.2 Yeast Influence on Higher Alcohols

Higher or fusel alcohols are one of the most significant groups of volatile compounds formed by the action of yeast enzymes. By definition, they are alcohols which contain more than two C-atoms. This definition includes the branched-chain alcohols such as isobutanol, amyl alcohol, isoamyl alcohol, and 2-phenylethanol. Higher alcohols are derivatives of amino acids. The biosynthesis occurs via Ehrlich pathway. This pathway includes different transamination, decarboxylation, and oxidoreduction reactions catalyzed by at least three aminotransferases, five decarboxylases, and six dehydrogenases. The first reaction in Ehrlich pathway is transamination of appropriate amino acid and formation of the corresponding α-keto-acid. Conversion of the resulting α-keto-acid to the branched-chain aldehyde with one carbon-less atom is catalyzed by pyruvate decarboxylase. In the last step, a reduction of the branched-chain aldehyde to corresponding higher alcohol occurs. This NADH-dependent reaction is catalyzed by the alcohol dehydrogenase (Fig. 6.3). Amino acids valine, leucine, and isoleucine are precursors of formation branched-chain aliphatic alcohols isobutanol, amyl alcohol, and isoamyl alcohol, respectively. The precursor of aromatic alcohols 2-phenylethanol, tyrosol, and tryptophol are phenylalanine, tyrosine, and tryptophan, respectively (Hazelwood et al., 2008; Ugliano and Henschenke, 2009).

Major higher alcohols (isobutanol, 1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-phenylethanol) are produced by yeasts during the fermentation process, and because of that, the concentrations of higher alcohols in wines are one of the important variables for differentiating between yeast strains. The principal compound among them is 3-methyl-1-butanol that together with others, in moderate concentrations can have the positive influence on wine complexity and aroma. In fact, total alcohol concentrations exceeding 400 mg/L, mainly connected with higher isoamyl alcohol content can have a negative effect, but lower concentrations impart positive fruity characters (Lambrechts and Pretorius, 2000). Influence and characterization of 52 *S. cerevisiae* strains on the volatile compounds production were done by Romano et al. (2003). There was no marked difference in the contents of 1-propanol production, but significant strain variability was noticed in the concentrations of isobutanol and isoamyl alcohol. Opposite to that among 59 *H. uvarum* tested strains showed variability in all produced higher alcohols except in isobutanol. Also, when compared to *S. cerevisiae* strains, *H. uvarum* strains tested gave lower concentrations of higher alcohols (Romano et al., 2003). Effect of the single culture of *H. osmophila*, *H. guilliermondii,* and *Pichia membranifaciens* compared to commercial *S. cerevisiae* strain showed some differences. All non-*Saccharomyces* yeasts tested produced the lower amount of total alcohols and relatively high concentrations of

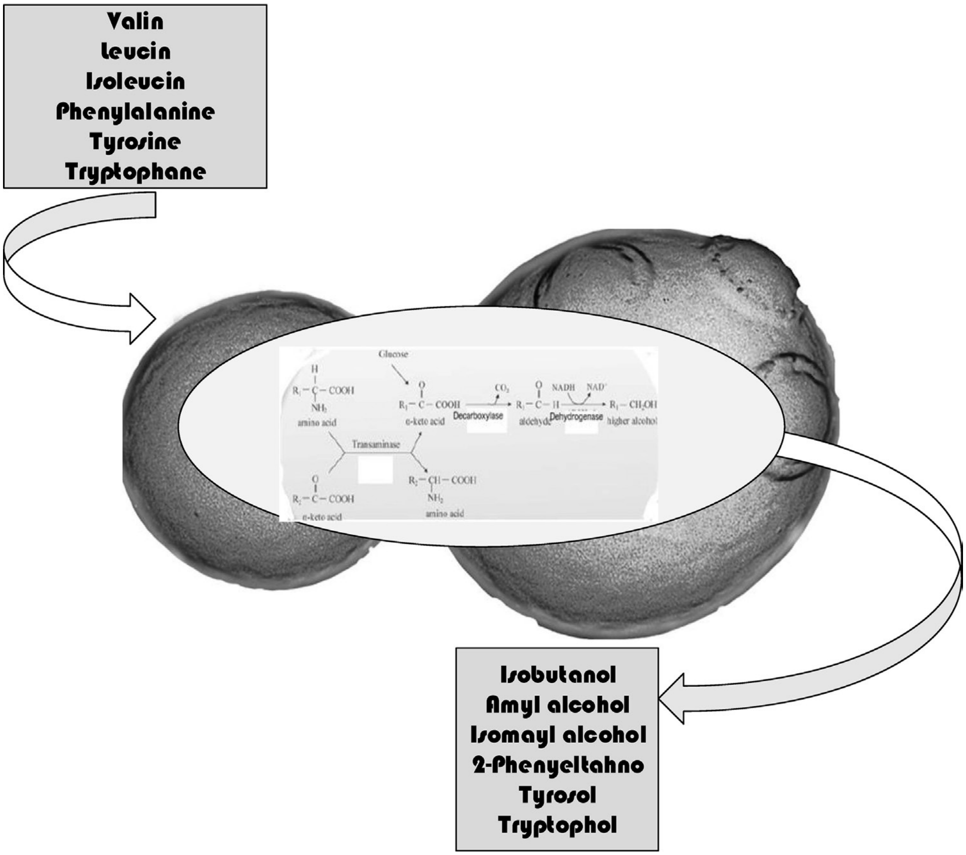

Fig. 6.3 Biosynthesis of higher alcohols from amino acids by *S. cerevisiae* enzymes.

2-phenylethanol and isoamyl alcohol, especially *H. osmophila* (Viana et al., 2008). A pure culture of *H. uvarum* and *H. guilliermondii* gave wines with a significantly lower concentrations of total alcohols as well as 3-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol in comparison to the wines obtained by a pure culture of *S. cerevisiae*. The lowest content in 1-propanol was obtained in wines obtained by a pure culture of *H. uvarum* while the wines produced by *S. cerevisiae*, in pure or mixed cultures, presented higher concentrations of 2-phenylethanol (Moreira et al., 2008). Similar results were also presented by Rojas et al. (2003), where two non-*Saccharomyces* wine yeast strains, *H. guilliermondii* and *Pichia anomala* were tested in wine fermentations as pure and mixed cultures together with *S. cerevisiae*. Obtained results showed that the highest amount of 2-phenylethanol founded in wines produced by *S. cerevisiae* followed by mixed cultures of *S. cerevisiae* and *H. guilliermondii* and *P. anomala* while the pure culture of *H. guilliermondii* produced the lowest content. In the study by Lage et al. (2014) single and mixed culture of *H. guilliermondii* and *S. cerevisiae* were used to ferment natural grape juice. *H. guilliermondii* single-culture wines contained the lowest amount of higher alcohols as well as 2-phenylethanol what is in agreement with the data presented by Rojas et al. (2003). The content of isobutanol was higher compared to mixed and pure *S. cerevisiae* culture. Medina et al. (2013) found out that cofermentation with *H. vineae* and *S. cerevisiae* resulted in significant decrease in higher alcohols, including 2-phenylethanol, 1,3 propandiol, 3-methyl 1-propanol, and tyrosol. In the study by Tristezza et al. (2016), the use of the apiculate yeast *H. uvarum* in mixed starter culture with *S. cerevisiae* decreased the total higher alcohol content and resulted in a concentration of 2-phenylethanol just above its sensory threshold. Callejon et al. (2010) tested the influence of five *S. cerevisiae* yeast strains (four autochthonous and one commercial) on the volatile compounds production and noted that the concentration of higher alcohols significantly differs. The highest total contents, as well as 3-methyl-1-butanol and 2-phenylethanol concentrations, were found in two wines produced by autochthonous *S. cerevisiae* yeasts. Same results were achieved in the research by Torrens et al. (2008) where compounds like isobutanol and isoamyl alcohol were among higher alcohols quantitatively the most representative ones with values varied significantly depending on the *S. cerevisiae* strain used. According to Torrens et al. (2008), alcohols characterized by a "vegetal" and "herbaceous" aroma, such as 1-hexanol and cis-3-hexen-1-ol, even though it is well known that they are formed by enzyme action in the prefermentation stage (Pérez-Coello et al., 1999), also appeared to be linked to the yeast strain used what was also confirmed by Callejon et al. (2010). Concentrations of higher alcohols in Chardonnay wines fermented with six commercial *S. cerevisiae* yeast strains, two individual Burgundian, and four mixed Burgundian *S. cerevisiae* strains were presented in the work by Saberi et al. (2012). In their research, mixed Burgundian strains produced similar amounts of some higher alcohols (2-methyl-1-butanol, 3-methyl-1-butanol) compared to the individual Burgundian strains but higher concentrations of 2-methyl-1-butanol and lower concentrations of 1-propanol and 2-phenylethanol compared to industrial strains. The mixed Burgundian strains produced moderate levels of *n*-butanol and isobutanol compared to individual Burgundian strains. According to Saberi et al. (2012), these different patterns in the alcohol production by mixed Burgundian strains compared to the individual Burgundian and industrial strains suggested that the mixed yeasts may have metabolic interactions with one another, allowing them to respond differently to the different substrates. Similar research was done by Gustafsson et al. (2016) where fermentation treatments with two commercial *S. cerevisiae* strains used as a pure culture controls and coinoculated treatments showed significant differences in the amount of higher alcohol produced. One of the tested *S. cerevisiae* strains was the strong producer of isobutanol, alone and in coinoculated treatment. Production of isoamyl alcohol had the same trend as isobutanol, concerning pure cultures while the coinoculations did not outcome in a notable trend. Despite, the chemical composition of the coinoculations had some of the characteristics of both pure culture fermentations, which affirms the results of other studies demonstrating that chemical profiles vary between coinoculation and pure culture fermentations (King et al., 2010; Saberi et al., 2012) and indicating that the synergy between two or more *Saccharomyces* strains can produce a new chemical profile not identified in the pure cultures. The use of *P. fermentans* in pure cultures and sequential mixtures with *S. cerevisiae* was studied by Clemente-Jimenez et al. (2005) who demonstrated that must fermentation with *P. fermentans* in sequential mixtures increased the concentrations of 1-propanol, *n*-butanol, and 1-hexanol. Andorrà et al. (2010) used several combinations of the commercial strain of *S. cerevisiae* and the strains *C. zemplinina* and *H. uvarum* previously isolated from wine fermentations. *S. cerevisiae* was the lowest producer of higher alcohols, whereas *C. zemplinina* was the highest. The total higher alcohol amount in the mixed fermentations was between those of the pure *S. cerevisiae* and *C. zemplinina* fermentations, although levels were closer to those of *S. cerevisiae*. The strong difference between *C. zemplinina* and *S. cerevisiae* was due to a significant increase in 1-propanol, 2-methyl-1-propanol, isoamyl alcohol, and 2-phenylethanol, whereas the differences between mixed fermentations were mostly due to the increases in 2-phenylethanol and 2-methyl-1-propanol. Two years latter Andorrà et al. (2012) explored the effect of pure and mixed inocula on synthetic grape must fermentation by using *S. cerevisiae, H. uvarum,* and *C. zemplinina*. *C. zemplinina* proved to be a strong producer of higher alcohols, yet in this research primarily connected with the high production of isobutyl alcohol. While the pure cultures showed similar levels of isoamyl alcohol, the mixed cultures showed significantly lower levels of this compound. Also, no marked differences were detected in the amount of 2-phenylethanol produced by pure or mixed starters even though they were pointed out in the previous work by the same authors. Mixed-culture fermentation trials by Comitini et al. (2011) were carried out by inoculating non-*Saccharomyce*s yeasts *C. zemplinina*, *L. thermotolerans* (synonym *K. thermotolerans*), *T. delbrueckii*, and *M. pulcherrima* in combination with the *S. cerevisiae* as a starter. Results of the higher alcohol compounds showed that all of the wines produced by *C. zemplinina* and *S. cerevisiae* did not show meaningful differences with those obtined by pure cultures of *S. cerevisiae*. On the contrary, other non-*Saccharomyces* yeasts used positively influenced the production of 2-phenylethanol, among them mixed cultures with *L. thermotolerans* resulted in the highest productions of this compound. Sadoudi et al. (2012) also used *C. zemplinina*, *T. delbrueckii*, and *M. pulcherrima* to conduct fermentations either in monoculture or in coculture with *S. cerevisiae*. Contrary to data presented by Andorrà et al. (2010, 2012) significantly the lowest amount of total higher alcohol was produced by pure culture of *C. zemplinina* whose concentration was 2.5 times lower compared to *S. cerevisiae*. Differences in the production of higher alcohols by single cultures of *M. pulcherrima* and *T. delbrueckii* compared to *S. cerevisiae* were not so distinguished but a significant increase in the production of higher alcohols was observed in the *M. pulcherrima/S. cerevisiae and T. delbrueckii/S. cerevisiae* coculture primarily linked to the higher production of 2-phenylethanol. The work by Loira et al. (2014) examined the fermentative behavior of five strains of *T. delbrueckii* in sequential fermentations with *S. cerevisiae* showing that the sequential fermentations yielded smaller concentrations of 3-methyl-1-butanol and higher concentrations of 1-propanol than the single-culture *S. cerevisiae* fermentation, and similar or smaller amounts of 2-methyl-1-butanol depending on the strain. Defining the impact and the contribution of starter cultures of *T. delbrueckii*, inoculated sequentially with *S. cerevisiae* Azzolini et al. (2015) showed that the increase in 2-phenylethanol was a clear effect of the copresence of *T. delbrueckii* and *S. cerevisiae* yeasts as previously also reported by Sadoudi et al. (2012) and Cordero-Bueso et al. (2013). The differences in the 2-phenylethanol content between the wines fermented by two *T. delbrueckii* yeasts could be strain related, although Renault et al. (2009) did not find significant differences among several *T. delbrueckii* strains on the production of this alcohol. Influence of *Schizosaccharomyces pombe* as the sole fermentative yeast and in the mixed and sequential fermentations with *S. cerevisiae* showed moderate production of main higher alcohols (isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethanol), but a little bit higher by single *S. cerevisiae* yeast fermentation (Benito et al., 2014). These results were confirmed in a study by Benito et al. (2016a) where higher alcohols were synthetized in higher total amounts by *S. cerevisiae* fermentations compared to tested *S. pombe* strains used. Some differences were also noted between *S. pombe* strains in the production of 1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol. Cañas et al. (2011) examined the influence of sequential inoculation of *H. anomala* and *T. delbrueckii* with commercial *S. cerevisiae* strain in fermentation of Airén grape must in two production years. In the case of higher alcohols, in both vintages there was a decrease in the content of these compounds in wines inoculated with *H. anomala*. In wines inoculated with *T. delbrueckii*, the decline was smaller in the 2007 vintage, whereas in the 2008 vintage there was slight increases. When non-*Saccharomyces* strains were used, there was a significant decrease, more pronounced in wines inoculated with *H. anomala*, in the concentration of C_6 alcohols in both vintages. Research by the same authors (Cañas et al., 2014) on the influence of sequential inoculation of *W. anomalus* (synonym *H. anomala*) and *S. cerevisiae* strain in red wine production confirmed the decline in C_6 alcohols concentrations in wines inoculated by *W. anomalus*. Higher alcohol concentrations measured after fermentation of single-culture *S. cerevisiae* and sequential fermentation with *L. thermotolerans, P. kluyveri*, and *M. pulcherrima* pointed out *S. cerevisiae* as the stronger producer in general, while *L. thermotolerans* was the second-best producer of 2-phenylethanol. Among non-*Saccharomyces* fermentations, there were no marked differences in the production of higher alcohols (Benito et al., 2015; Table 6.5).

6.3.3 Yeast Influence on Esters

Esters are a large class of compounds that contribute to the fruity notes of wine. Based on the chemical structure they can be divided into acetate esters and ethyl fatty acid esters. Among acetate ester isobutyl acetate, active amyl acetate, isoamyl acetate, hexyl acetate, and 2-phenylethyl acetate are the most important ones. Ethyl fatty acid esters contain ethanol as alcoholic part and fatty acid (from C3 up to C12) as acid part of the ester. Ethyl hexanoate, ethyl octanoate, and ethyl decanoate are the most abundant ethyl fatty acid esters in wine. Ethyl 2-methylpropanoate, ethyl-2-methylbutanoate, and ethyl-3-methylbutanoate are branched-chain esters with very low odor threshold values, but in comparison with acetate esters and ethyl fatty acid esters, they are present in much lower concentrations. Yeast enzymes alcohol acetyltransferases condense a particular higher alcohol and acetyl-CoA and release corresponding acetate ester. There is a little knowledge about enzymology and genetics of ethyl fatty acid esters formation during fermentation. Nowadays it is considered that the majority of ethyl fatty acid esters to be formed by enzyme action through esterification of activated fatty acids (acyl-CoA). One of the possible confirmation of the above-mentioned assumptions is the recent identification of two enzymes responsible for the biosynthesis of ethyl esters of medium-chain fatty acids (Swiegers et al., 2005).

More then 160 esters have been identified in wine (Jackson, 2008) forming one of the most significant groups of aroma compounds. Among them, C_4-C_{10} ethyl esters of organic acids, ethyl esters of straight-chain fatty acids, and acetates of higher alcohols are largely responsible for the fruity aroma of wine (Ebeler, 2001), particularly in young wines. The above-mentioned esters can positively affect on wine quality, especially in wine from cultivars with neutral flavors that

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
S. cerevisiae	29.13	163.85	23.80	1.93	17.84
S. cerevisiae	24.17	142.19	19.69	1.51	14.24
T. delbrueckii + *S. cerevisiae*	22.31	105.02	21.21	0.64	21.34
T. delbrueckii + *S. cerevisiae*	19.68	87.45	16.55	0.60	19.17
S. cerevisiae	na	182	na	1.5	21.3
T. delbrueckii	na	149	na	1.2	15.5
K. marxianus	na	152	na	1.3	25.0
S. pombe	5.13	11.36	23.34	1.31	21.76
S. pombe	5.45	12.16	24.48	1.39	22.28
S. pombe	5.36	11.98	25.68	1.36	21.44
S. pombe	5.28	12.28	24.19	1.34	22.35
S. pombe	7.23	18.44	31.08	1.42	24.17
S. pombe	7.44	18.88	33.16	1.46	24.66
S. cerevisiae	7.81	26.12	40.44	2.45	28.97
S. cerevisiae	7.96	28.06	42.83	2.48	29.12
S. cerevisiae	na	280.7	47	na	23.3
S. cerevisiae	na	249.7	53.7	na	22.1
S. cerevisiae	na	254.7	45.3	na	27.3
S. cerevisiae	na	261.3	51.3	na	18.4
S. cerevisiae	na	132.82	na	0.96	18.16
L. thermotolerans + *S. cerevisiae*	na	126.92	na	1.02	19.32
L. thermotolerans + *S cerevisiae*	na	102.43	na	0.98	22.08
S. cerevisiae	na	171.11	24.02	0.90	22.23
Z. florentina	na	57.82	36.85	1.55	23.53
Z. florentina + *S. cerevisiae*	na	126.74	33.18	1.15	39.97
Z. florentina + *S. cerevisia*	na	98.96	35.32	1.07	32.30
Z. florentina + *S. cerevisiae*	na	105.95	36.97	1.19	32.07

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	na	na	na	na	na	Belda et al. (2017)
na	na	na	na	na	na	Belda et al. (2017)
na	na	na	na	na	na	Belda et al. (2017)
na	na	na	na	na	na	Belda et al. (2017)
2.0	38	na	na	na	na	Vigentini et al. (2016)
1.3	27	na	na	na	na	Vigentini et al. (2016)
1.4	38	na	na	na	na	Vigentini et al. (2016)
na	9.31	11.42	na	na	na	Benito et al. (2014)
na	8.57	10.12	na	na	na	Benito et al. (2014)
na	9.86	13.26	na	na	na	Benito et al. (2014)
na	8.94	10.73	na	na	na	Benito et al. (2014)
na	12.11	18.99	na	na	na	Benito et al. (2014)
na	13.52	19.76	na	na	na	Benito et al. (2014)
na	17.51	28.45	na	na	na	Benito et al. (2014)
na	18.13	31.33	na	na	na	Benito et al. (2014)
na	270.7	47.3	na	na	na	Gustafsson et al. (2016)
na	89	34	na	na	na	Gustafsson et al. (2016)
na	173	41	na	na	na	Gustafsson et al. (2016)
na	138.3	39	na	na	na	Gustafsson et al. (2016)
na	11.36	na	na	na	na	Benito et al. (2016a)
na	12.56	na	na	na	na	Benito et al. (2016a)
na	14.42	na	na	na	na	Benito et al. (2016a)
na	108.66	27.44	na	na	na	Lencioni et al. (2016)
na	17.50	28.66	na	na	na	Lencioni et al. (2016)
na	44.68	20.40	na	na	na	Lencioni et al. (2016)
na	31.40	35	na	na	na	Lencioni et al. (2016)
na	22.95	34.09	na	na	na	Lencioni et al. (2016)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	**1-Butanol**	**3-Methylbutan-1-ol**	**2-Methylbutan-1-ol**	**1-Hexanol**	**2-Phenylethanol**
H. vineae[a]	58	36,361	na	386	8099
S. cerevisiae[a]	84	61,355	na	328	16,830
S. pombe	22.16	14.47	25.84	4.22	34.81
S. pombe	15.49	13.63	28.02	3.79	37.34
S pombe	13.51	28.13	111.48	na	na
S. pombe	8.09	27.92	111.62	na	na
S. cerevisiae	18.09	59.20	257.40	na	na
H. uvarum + *S. cerevisiae*[a]	106	14,785	na	492	11,577
H. uvarum+*S. cerevisiae*[a]	–	13,968	na	491	12,962
S. cerevisiae[a]	178	15,754	na	776	13,760
S. cerevisiae	0.35	na	205.55	na	83.75
M. pulcherrima + *S. cerevisiae*	0.45	na	129.12	na	70.83
S. uvarum + *S. cerevisiae*	0.62	na	159.49	na	206.31
M. pulcherrima + *S. uvarum* + *S. cerevisiae*	0.61	na	172.27	na	194.78
S. cerevisiae	0.38	na	140.82	na	39.93
M. pulcherrima + *S. cerevisiae*	0.38	na	103.31	na	75.93
S. uvarum + *S. cerevisiae*	0.61	na	194.80	na	158.27
M. pulcherrima + *S. uvarum* + *S. cerevisiae*	0.55	na	164.34	na	169.94
S. bayanus	na	119	na	na	6.37
S. cerevisiae	na	174	na	na	10.07
S. cerevisiae	0.51	178.6	na	0.92	53.6
T. delbrueckii + *S. cerevisiae*	0.64	213.9	na	0.77	44.9
M. pulcherrima + *S. cerevisiae*	0.52	231	na	0.92	48.2
S. bayanus	14.96	151.37	16.54	1.1	24.67

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
321	2388	na	7	120	na	Lleixà et al. (2016)
599	1895	na	129	na	na	Lleixà et al. (2016)
na	8.02	28.48	na	na	38.85	Mylona et al. (2016)
na	8.51	29.41	na	na	40.92	Mylona et al. (2016)
na	31.47	23.72	na	na	41.64	Mylona et al. (2016)
na	30.43	24.85	na	na	41.62	Mylona et al. (2016)
na	120.60	28.21	na	na	40.78	Mylona et al. (2016)
na	966	312	55	66	na	Tristezza et al. (2012)
na	701	na	79	80	na	Tristezza et al. (2012)
na	1427	211	81	56	na	Tristezza et al. (2012)
na	152.38	na	na	na	na	Varela et al. (2016)
na	222.01	na	na	na	na	Varela et al. (2016)
na	81.71	na	na	na	na	Varela et al. (2016)
na	87.94	na	na	na	na	Varela et al. (2016)
na	243.52	na	na	na	na	Varela et al. (2016)
na	215.40	na	na	na	na	Varela et al. (2016)
na	171.75	na	na	na	na	Varela et al. (2016)
na	160.42	na	na	na	na	Varela et al. (2016)
na	19.1	39	na	na	na	Mouret et al. (2015)
na	29.8	34.7	na	na	na	Mouret et al. (2015)
na	22.2	na	na	0.55	na	González-Royo et al. (2015)
na	32.8	na	na	0.54	na	González-Royo et al. (2015)
na	29.9	na	na	0.54	na	González-Royo et al. (2015)
na	na	na	na	na	na	Benito et al. (2014)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
K. thermotolerans + *S. bayanus*	17.55	129.41	17.12	1.2	21.34
P. kluyveri + *S. bayanus*	12.2	127.52	15.63	0.54	20.32
M. Pulcherrima + *S. bayanus*	9.69	117.29	14.63	0.72	19.10
S. cerevisiae	1.15	297	na	1.59	21.5
W. anomalus + *S. cervisiae*	1.16	324	na	1.48	21.0
T. delbrueckii + *S. cerevisiae*	4.2	40.3	206	8.1	34.5
T. delbrueckii + *S. cerevisiae*	4.4	51.4	232	7.7	48
T. delbrueckii + *S. cerevisiae*	4.4	44.2	225.2	7.6	52.3
T. delbrueckii + *S. cerevisiae*	4.2	36.4	209.8	7.6	43.1
T. delbrueckii + *S. cerevisiae*	4.4	32.7	178	7.1	37.1
S. cerevisiae	4.2	60.3	212.3	6.5	101.8
S. uvarum	6.3	66.4	222.4	6.2	55.4
S. pombe	4.39	50.09	85.80	4.89	15.37
S. pombe + *S. cerevisiae* mix	4.60	50.18	80.05	4.68	19.33
S. pombe + *S. cerevisiae* seq	4.20	44.38	72.97	4.68	18.85
S. cerevisiae	4.39	55.34	99.56	5.22	22.55
S. cerevisiae[a]	na	na	na	595.6	37,197.0
T. delbrueckil1 + *S. cerevisiae*[a]	na	na	na	613.7	60,309.5
T. delbrueckl2 + *S. cerevisiae*[a]	na	na	na	658.0	45,604.6
S. cerevisiae[a]	na	na	na	1575.9	36,443.2

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	na	na	na	na	na	Benito et al. (2014)
na	na	na	na	na	na	Benito et al. (2014)
na	na	na	na	na	na	Benito et al. (2014)
0.37	56.9	30.9	0.05	0.22	126	Cañas et al. (2014)
0.32	61.4	32.3	0.06	0.22	169	Cañas et al. (2014)
na	50.8	64	na	na	14.4	Loira et al. (2014)
na	58.4	68.4	na	na	14.3	Loira et al. (2014)
na	51.5	65.4	na	na	12.7	Loira et al. (2014)
na	51.8	58.4	na	na	11.7	Loira et al. (2014)
na	39.1	46.8	na	na	10.6	Loira et al. (2014)
na	22.9	25	na	na	13.4	Loira et al. (2014)
na	65.6	31.7	na	na	12.4	Loira et al. (2014)
na	na	13.37	na	na	13.07	Benito et al. (2014)
na	na	15.42	na	na	15.50	Benito et al. (2014)
na	na	13.34	na	na	15.83	Benito et al. (2014)
na	na	17.23	na	na	17.11	Benito et al. (2014)
1480.4	na	na	33.8	9.7	na	Azzolini et al. (2015)
1719.3	na	na	39.7	11.2	na	Azzolini et al. (2015)
1549.2	na	na	36.8	10.2	na	Azzolini et al. (2015)
878.6	na	na	28.9	25.8	na	Azzolini et al. (2015)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
T. delbruecki1+ *S. cerevisiae*[a]	na	na	na	1857.9	57,035.1
T. delbruecki2 + *S. cerevisiae*[a]	na	na	na	2014.2	41,586.8
S. cerevisiae	na	291.61	104.96	na	115.59
H. guilliermondii + *S. cerevisiae*	na	251.79	103.74	na	64.21
H. guilliermondii	na	82.41	53.72	na	42.82
S. cerevisiae	na	123.85	na	6.08	74.56
S. cerevisiae	na	143.65	na	4.82	29.35
S. cerevisiae	na	115.65	na	5.33	53.94
S. cerevisiae	na	113.48	na	3.98	56.77
H. vineae + *S. cerevisiae*[a]	189	54.3	na	858	13.55
S. cerevisiae- spontaneous[a]	75	50.33	na	506	40.95
S. cerevisiae[a]	149	63.2	na	607	32.89
S. cerevisiae	na	171.11	24.02	0.90	22.23
L. thermotolerans	na	96.57	25.47	1.22	18.93
S. cerevisiae + *L. thermotolerans*	na	154.35	44.07	0.86	31.62
S. cerevisiae + *L. thermotolerans*	na	125.62	36.84	0.77	25.33
S. cerevisiae + *L. thermotolerans*	na	137.19	35.07	0.81	30.15
S. cerevisiae	2.8	109.2	na	1.3	na
M. pulcherrima + *S. cerevisiae*	2.4	122.6	na	0.7	na
S. cerevisiae	1.5	156.6	na	1.3	na
M. pulcherrima + *S. cerevisiae*	2.0	219.8	na	1.5	na
S. cerevisiae	0.04	5.78	1.15	1.91	0.90
S. cerevisiae	0.04	7.16	1.17	1.94	1.25
S. cerevisiae	0.05	7.05	1.42	2.2	1.24

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
1527.7	na	na	28.6	40.1	na	Azzolini et al. (2015)
989.8	na	na	27.6	35.0	na	Azzolini et al. (2015)
na	49.81	14.85	na	na	na	Lage et al. (2014)
na	74.09	21.84	na	na	na	Lage et al. (2014)
na	127.63	30.34	na	na	na	Lage et al. (2014)
na	128.71	na	0.047	na	na	Furdíková et al. (2014)
na	139.38	na	0.019	na	na	Furdíková et al. (2014)
na	132.52	na	0.017	na	na	Furdíková et al. (2014)
na	107.17	na	0.020	na	na	Furdíková et al. (2014)
na	na	na	na	na	na	Medina et al. (2013)
na	na	na	na	na	na	Medina et al. (2013)
na	na	na	na	na	na	Medina et al. (2013)
na	108.66	27.44	na	na	na	Gobbi et al. (2013)
na	13.47	54.84	na	na	na	Gobbi et al. (2013)
na	24.33	39.52	na	na	na	Gobbi et al. (2013)
na	13.38	68.99	na	na	na	Gobbi et al. (2013)
na	15.72	62.21	na	na	na	Gobbi et al. (2013)
na	79.5	na	na	na	na	Contreras et al. (2014)
na	252.5	na	na	na	na	Contreras et al. (2014)
na	60.5	na	na	na	na	Contreras et al. (2014)
na	134.6	na	na	na	na	Contreras et al. (2014)
na	6.66	5.03	na	na	na	Saberi et al. (2012)
na	9.74	2.71	na	na	na	Saberi et al. (2012)
na	7.4	2.11	na	na	na	Saberi et al. (2012)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
S. cerevisiae	0.03	6.72	1.18	1.89	1.07
S. cerevisiae	0.1	6.60	1.24	1.81	0.87
S. cerevisiae	0.04	9.29	1.76	1.60	1.65
S. cerevisiae[a]	na	46,744	2505	965.5	32,784
M. pulcherrima[a]	na	32,342	1093	1345	39,908
T. delbrueckii[a]	na	33,511	3510	768.6	28,504
C. zemplinina[a]	na	11,660	3219	795.1	24,628
C. zemplinina + *S. cerevisiae*[a]	na	35,694	1926	1208	37,035
M. pulcherrima + *S. cerevisiae*[a]	na	48,095	2368	690.1	48,751
T. delbruecki + *S. cerevisiae*[a]	na	36,620	1125	1373	47,126
H. vineae + *S. cerevisiae* simulteneous	na	na	na	na	16
H. vineae + *S. cerevisiae* sequential	na	na	na	na	18.2
H. osmophila + *S. cerevisiae*	na	127.95	21.55	0.33	6.85
P. anomala + *S. cerevisiae*	na	106.29	23.31	0.74	8.20
S. ludwigii + *S. cerevisiae*	na	133.81	34.92	1.33	33.58
Z. florentinus + *S. cerevisiae*	na	113.40	24.78	2.43	39.39
S. cerevisiae	na	113.21	12.33	0.35	13.00
S. cerevisiae	na	156	63.2	na	21.2
H. guilliermondii	na	154	59.8	na	22.3
C. pulcherrima	na	14.21	na	0.19	5.37
S. cerevisiae	na	46.44	na	0.31	3.66
C. pulcherrima + *S. cerevisiae* simulaneous	na	41.78	na	0.21	3.57
C. pulcherrima + *S. cerevisiae* sequential	na	71.61	na	0.18	9.46
S. cerevisiae	na	120.29	30.15	na	na

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	12.8	1.42	na	na	na	Saberi et al. (2012)
na	11.8	1.61	na	na	na	Saberi et al. (2012)
na	15.2	2.01	na	na	na	Saberi et al. (2012)
na	2505	na	12.15	77.19	na	Sadoudi et al. (2012)
na	1093	na	19.16	99.29	na	Sadoudi et al. (2012)
na	3510	na	15.88	78.27	na	Sadoudi et al. (2012)
na	3219	na	19.32	66.14	na	Sadoudi et al. (2012)
na	1926	na	15.19	90.98	na	Sadoudi et al. (2012)
na	2368	na	14.79	76.95	na	Sadoudi et al. (2012)
na	1125	na	19.32	111.6	na	Sadoudi et al. (2012)
na	38	1.15	na	na	na	Viana et al. (2011)
na	48	0.60	na	na	na	Viana et al. (2011)
na	83.15	49.61	na	na	na	Domizio et al. (2011)
na	83.59	76.85	na	na	na	Domizio et al. (2011)
na	89.24	37.94	na	na	na	Domizio et al. (2011)
na	83.96	97.02	na	na	na	Domizio et al. (2011)
na	77.17	37.88	na	na	na	Domizio et al. (2011)
na	81.7	62.5	na	na	na	Moreira et al. (2011)
na	86.5	73.0	na	na	na	Moreira et al. (2011)
na	22.54	na	na	na	na	Rodriguez et al. (2010)
na	11.90	na	na	na	na	Rodriguez et al. (2010)
na	13.34	na	na	na	na	Rodriguez et al. (2010)
na	67.01	na	na	na	na	Rodriguez et al. (2010)
na	36.13	21.86	na	na	na	Erten and Tanguler (2010)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
W. saturnus + *S. cerevisiae*	na	130.55	32.87	na	na
W. saturnus + *S. cerevisiae*	na	135.31	34.35	na	na
W. saturnus + *S. cerevisiae*	na	132.13	33.44	na	na
S. cerevisiae	na	167.52	na	na	30.63
C. zemplina	na	334.63	na	na	227.9
C. zempina + *S. cerevisiae*	na	213.13	na	na	118.15
H. uvarum + *S. cerevisiae*	na	202.95	na	na	42.72
C. zemplina + *H. uvarum* + *S. cerevisiae*	na	199.96	na	na	61.22
T. delbrueckii	na	na	na	na	24.54
S. cerevisiae[a]	na	134,598.9	20,311.0	1456.3	12,648.2
S. cerevisiae[a]	na	93,584.2	18,255.8	1010.9	15,362.5
S. cerevisiae[a]	na	125,661.9	17,174.6	1595.0	14,170.1
S. cerevisiae[a]	na	99,056.8	21,381.7	1130.0	12,527.5
S. cerevisiae[a]	na	137,185.4	24,624.4	1310.4	14,247.7
S. cerevisiae[a]	na	94,932.8	13,950.1	1687.9	13,785.6
S. cerevisiae[a]	na	108,961.1	23,073.1	1583.0	11,929.4
S. cerevisiae	na	291.5	na	na	114.9
H. osmophila + *S. cerevisiae* (5:95)	na	256.6	na	na	95.2
H. osmophila + *S. cerevisiae*(10:90)	na	227.4	na	na	80.5
H. osmophila + *S. cerevisiae* (25:75)	na	202.8	na	na	83.5
H. osmophila + *S. cerevisiae* (50:50)	na	192.8	na	na	63.0
H. osmophila + *S. cerevisiae* (75:25)	na	202.0	na	na	46.6
H. osmophila + *S. cerevisiae* (90:10)	na	217.4	na	na	25.5

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	37.48	23.55	na	na	na	Erten and Tanguler (2010)
na	36.52	23.15	na	na	na	Erten and Tanguler (2010)
na	37.95	24.20	na	na	na	Erten and Tanguler (2010)
na	24.51	9.55	na	na	na	Andorrà et al. (2010)
na	468.86	30.58	na	na	na	Andorrà et al. (2010)
na	93.46	16.94	na	na	na	Andorrà et al. (2010)
na	55.39	11.47	na	na	na	Andorrà et al. (2010)
na	77.4	21.0	na	na	na	Andorrà et al. (2010)
na	17.52	na	na	na	na	Renault et al. (2015)
na	15,790.4	na	na	na	na	Swiegers et al. (2009)
na	12,066.1	na	na	na	na	Swiegers et al. (2009)
na	12,110.6	na	na	na	na	Swiegers et al. (2009)
na	12,864.9	na	na	na	na	Swiegers et al. (2009)
na	22,386.2	na	na	na	na	Swiegers et al. (2009)
na	12,737.4	na	na	na	na	Swiegers et al. (2009)
na	13,624.1	na	na	na	na	Swiegers et al. (2009)
na	40.3	5.8	na	na	na	Viana et al. (2009)
na	44.0	4.8	na	na	na	Viana et al. (2009)
na	47.9	3.9	na	na	na	Viana et al. (2009)
na	52.1	4.5	na	na	na	Viana et al. (2009)
na	56.9	3.8	na	na	na	Viana et al. (2009)
na	58.4	3.7	na	na	na	Viana et al. (2009)
na	60.3	3.0	na	na	na	Viana et al. (2009)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
S. cerevisiae	na	140.7	na	0.89	10.58
S. cerevisiae	na	154.2	na	0.84	13.18
S. cerevisiae	na	152.8	na	0.78	11.81
S. cerevisiae	na	146.7	na	0.91	12.60
S. cerevisiae spont.	na	173.8	na	0.94	13.94
H. uvarum	na	53.7	26.0	na	34.5
H. guilliermondii	na	46.7	50.4	na	32.5
S. cerevisiae	na	205	127	na	58.1
H. uvarum + *S. cerevisiae*	na	186	125	na	56.3
H. guilliermondii + *S. cerevisiae*	na	159	102	na	43.1
H. uvarum + *H. guilliermondii* + *S. cerevisiae*	na	147	114	na	55.8
S. cerevisiae	na	190	na	na	30.7
H. guilliermondii	na	30.6	na	na	27.5
H. guilliermondii	na	21.2	na	na	11.5
H. osmophila	na	89.6	na	na	23.8
P. membranifaciens	na	22.4	na	na	15.7
S. cerevisiae spontaneous	na	142.83	34.24	na	na
S. cerevisiae 1×10^4	na	178.96	44.83	na	na
S. cerevisiae 1×10^5	na	163.92	35.33	na	na
S. cerevisiae 1×10^6	na	213.83	50.86	na	na
S. cerevisiae 1×10^7	na	255.70	55.16	na	na
S. cerevisiae	na	265.38	na	na	82.09
H. guilliermondii	na	99.76	na	na	14.74
P. anomala	na	83.52	na	na	30.50
S. cerevisiae + *H. guilliermondii*	na	167.53	na	na	29.18
S. cerevisiae + *P. anomala*	na	204.13	na	na	54.83
S. cerevisiae	2.20	166	na	2.23	19.2
S. cerevisiae	2.10	182	na	2.34	26.0
S. bayanus	1.52	202	na	2.40	38.0
H. uvarum	0.61	29.5	na	2.50	12.3

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	24.0	na	na	0.21	na	Torrens et al. (2008)
na	18.5	na	na	0.21	na	Torrens et al. (2008)
na	22.83	na	na	0.21	na	Torrens et al. (2008)
na	15.5	na	na	0.22	na	Torrens et al. (2008)
na	31.33	na	na	0.23	na	Torrens et al. (2008)
na	na	13.4	na	na	na	Moreira et al. (2008)
na	na	21.4	na	na	na	Moreira et al. (2008)
na	na	21.3	na	na	na	Moreira et al. (2008)
na	na	21.2	na	na	na	Moreira et al. (2008)
na	na	21.8	na	na	na	Moreira et al. (2008)
na	na	29.1	na	na	na	Moreira et al. (2008)
na	45.2	4.3	na	na	na	Viana et al. (2008)
na	43.4	7.2	na	na	na	Viana et al. (2008)
na	47.2	5.9	na	na	na	Viana et al. (2008)
na	43.1	10.8	na	na	na	Viana et al. (2008)
na	48.9	5.9	na	na	na	Viana et al. (2008)
na	36.39	18.79	na	na	na	Furdíková et al. (2014)
na	40.97	15.25	na	na	na	Furdíková et al. (2014)
na	33.44	12.34	na	na	na	Furdíková et al. (2014)
na	44.10	12.89	na	na	na	Furdíková et al. (2014)
na	60.65	14.88	na	na	na	Furdíková et al. (2014)
na	16.43	16.67	na	na	na	Rojas et al. (2003)
na	57.61	14.55	na	na	na	Rojas et al. (2003)
na	24	27.48	na	na	na	Rojas et al. (2003)
na	32.34	14.67	na	na	na	Rojas et al. (2003)
na	13.87	12.19	na	na	na	Rojas et al. (2003)
na	62.0	10.1	0.055	0.040	na	Gil et al. (1996)
na	44.7	12.1	0.050	0.050	na	Gil et al. (1996)
na	35.5	10.0	0.055	0.046	na	Gil et al. (1996)
na	9.80	3.70	0.050	0.040	na	Gil et al. (1996)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
K. apiculata	1.22	44.7	na	2.42	7.0
S. cerevisiae	na	5.71	na	0.14	7.08
Starm. bacillaris	na	3.90	na	0.19	4.46
S. cerevisiae + *Starm. bacillaris* coinoculation	na	6.46	na	0.19	7.46
S. cerevisiae + *Starm. bacillaris* sequential	na	7.93	na	0.20	10.11
S. cerevisiae[a]	2059	181,159	na	na	30,893
H. uvarum + *S. cerevisiae*[a]	1783	179,436	na	na	40,043
H. uvarum + *S. cerevisiae*[a]	2322	183,738	na	na	87,214
S. cerevisiae spontaneous	na	175.23	na	0.81	10.79
S. cerevisiae	na	182.20	na	0.95	9.29
S. cerevisiae	na	156.36	na	1.0	9.99
S. cerevisiae	na	153.3	25.3	2.1	23.3
S. cerevisiae	na	96.3	22.0	1.9	21.3
S. cerevisiae	na	113.3	22.7	2.0	24.4
S. cerevisiae	na	94.0	18	1.8	18.5
S. cerevisiae	na	108.7	25.3	2.1	23.4
S. cerevisiae	na	104	21.0	1.7	17.8
Saccharomyces spp.	na	105.7	21.3	2.0	20.2
Saccharomyces spp.	na	119.7	26.0	2.0	21.7
S. cerevisiae	na	96.7	22.3	1.7	20.8
S. cerevisiae	na	102.7	23.7	1.8	20.8
S. cerevisiae	na	85.7	19.7	1.6	21.4
S. cerevisiae	na	118.7	24.7	2.0	20.8
S. cerevisiae	na	127.0	22.0	1.9	23.8

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	18.0	9.70	0.050	0.045	na	Gil et al. (1996)
na	0.002	0.003	na	na	na	Englezos et al. (2016)
na	0.29	0.013	na	na	na	Englezos et al. (2016)
na	0.38	0.013	na	na	na	Englezos et al. (2016)
na	0.51	0.011	na	na	na	Englezos et al. (2016)
na	13,682	na	na	na	na	Hu et al. (2018)
na	11,956	na	na	na	na	Hu et al. (2018)
na	11,486	na	na	na	na	Hu et al. (2018)
na	37.39	35.02	na	na	na	Cordero-Bueso et al. (2016)
na	39.50	36.63	na	na	na	Cordero-Bueso et al. (2016)
na	23.52	25.80	na	na	na	Cordero-Bueso et al. (2016)
na	28.7	na	na	na	na	Srisamatthakarn (2011)
na	22.0	na	na	na	na	Srisamatthakarn (2011)
na	20.7	na	na	na	na	Srisamatthakarn (2011)
na	14.7	na	na	na	na	Srisamatthakarn (2011)
na	24.7	na	na	na	na	Srisamatthakarn (2011)
na	17.0	na	na	na	na	Srisamatthakarn (2011)
na	21.7	na	na	na	na	Srisamatthakarn (2011)
na	29.3	na	na	na	na	Srisamatthakarn (2011)
na	25.0	na	na	na	na	Srisamatthakarn (2011)
na	26.7	na	na	na	na	Srisamatthakarn (2011)
na	20.7	na	na	na	na	Srisamatthakarn (2011)
na	26.3	na	na	na	na	Srisamatthakarn (2011)
na	20.3	na	na	na	na	Srisamatthakarn (2011)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
W. anomalus	1.21	168.32	na	2.11	18.05
W. anomalus	1.55	163.53	na	2.06	20.34
K. aerobia	0.87	136.17	na	2.59	16.46
K. aerobia	1.02	119.66	na	2.75	15.46
W. anomalus + *S. cerevisiae*	1.01	172.25	na	2.16	19.14
W. anomalus + *S. cerevisiae*	1.11	162.48	na	2.02	21.29
K. aerobia + *S. cerevisiae*	0.80	142.37	na	2.47	15.44
K. aerobia + *S. cerevisiae*	1.04	149.44	na	2.32	16.51
S. cerevisiae	0.72	145.14	na	2.31	15.22
S. cerevisiae	na	41.74	na	na	12.53
C. zemplinina	na	44.69	na	na	15.40
C. zemplinina + *S. cerevisiae*	na	26.18	na	na	12.24
H. uvarum + *S. cerevisiae*	na	30.99	na	na	9.57
C. zemplinina + *H. uvarum* + *S. cerevisiae*	na	29.11	na	na	10.63
S. pombe + *S. cerevisiae* -mixed	0.52	na	na	1.68	19.50
S. pombe + *S. cerevisiae* mixed	0.57	na	na	1.44	22.15
S. pombe + *S. cerevisiae* mixed	0.41	na	na	1.48	22.70
T. delbrueckii + *S. cerevisiae* mixed	0.64	na	na	1.29	15.78
T. delbrueckii + *S. cerevisiae* mixed	0.73	na	na	1.42	21.66
T. delbrueckii + *S. cerevisiae* mixed	0.62	na	na	1.32	14.28
S. pombe + *S.cerevisiae*-seq	0.31	na	na	3.17	6.76

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	20.07	88.96	na	na	44.49	Lombard (2016)
na	20.42	87.73	na	na	43.90	Lombard (2016)
na	27.88	70.96	na	na	43.56	Lombard (2016)
na	22.55	65.01	na	na	42.37	Lombard (2016)
na	20.32	80.78	na	na	43.76	Lombard (2016)
na	20.85	81.35	na	na	44.25	Lombard (2016)
na	26.19	68.86	na	na	44.45	Lombard (2016)
na	23.92	82.01	na	na	45.01	Lombard (2016)
na	17.96	78.45	na	na	47.13	Lombard (2016)
na	14.47	44.23	na	na	na	Andorrà et al. (2012)
na	101.64	32.48	na	na	na	Andorrà et al. (2012)
na	20.66	46.02	na	na	na	Andorrà et al. (2012)
na	19.17	74.06	na	na	na	Andorrà et al. (2012)
na	17.05	67.64	na	na	na	Andorrà et al. (2012)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
S. pombe + *S. cerevisiae* seq	0.30	na	na	3.56	8.38
S. pombe + *S. cerevisiae* seq	0.28	na	na	3.32	8.50
T. delbrueckii + *S. cerevisiae* seq	1.31	na	na	2.38	24.62
T. delbrueckii + *S. cerevisiae* seq	3.09	na	na	2.41	20.16
T. delbrueckii + *S. cerevisiae* seq	0.86	na	na	2.20	20.86
S. cerevisiae	0.68	na	na	1.49	26.18
C. stellata	na	17.4	na	0.2	2.5
H. guilliermondii	na	34.5	na	0.3	5.2
L. fermentati	na	66.2	na	0.4	4.8
L. thermotolerans	na	19.7	na	1.1	7.0
P. kudriavzevii	na	22.6	na	0.4	1.9
P. membranifaciens	na	16.2	na	0.3	2.1
S. pombe	na	47.4	na	11.5	45.3
T. delbrueckii	na	146.1	na	0.9	12.0
W. anomalus	na	38.4	na	0.2	2.1
S. cerevisiae Vin7/*S. kudriavzev*	0.80	na	22	1.2	12

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	na	na	na	na	na	Loira et al. (2015)
na	30.1	37.8	na	na	na	Cordero-Bueso et al. (2013)
na	26.1	24.4	na	na	na	Cordero-Bueso et al. (2013)
na	37.1	19.2	na	na	na	Cordero-Bueso et al. (2013)
na	25.8	25.1	na	na	na	Cordero-Bueso et al. (2013)
na	32.2	47.2	na	na	na	Cordero-Bueso et al. (2013)
na	33.2	28.4	na	na	na	Cordero-Bueso et al. (2013)
na	17.8	18.4	na	na	na	Cordero-Bueso et al. (2013)
na	63.5	31.6	na	na	na	Cordero-Bueso et al. (2013)
na	32.8	43.1	na	na	na	Cordero-Bueso et al. (2013)
na	19	na	na	na	na	King et al. (2008)

Continued

Table 6.5 Dependence of Higher Alcohols Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	1-Butanol	3-Methylbutan-1-ol	2-Methylbutan-1-ol	1-Hexanol	2-Phenylethanol
S. cerevisiae QA23	1.0	na	15	1.2	13
S. cerevisiae Vin13	1.7	na	25	0.95	12
S. cerevisiae Vin7/*S. cerevisiae* QA23-coinoculation	1.0	na	18	1.2	12
S. cerevisiae Vin7/*S. cerevisiae* Vin13-coinoculation	1.4	na	24	0.98	11

na, not analyzed.
Results are expressed in mg/L except other is not indicate.
[a] Results are expressed in μg/L.

are consumed shortly after vinification (Lambrechts and Pretorius, 2000; Sumby et al., 2010). The synthesis of esters has been widely studied in *S. cerevisiae* during wine fermentation and various enzymes have been identified as playing a role in their formation. According to Torrens et al. (2008) and Tristezza et al. (2012), different strains of *S. cerevisiae* showed marked diversity in the ability to produce ethyl and acetate esters. Also when used in single and coinoculated fermentations different *S. cerevisiae* strains demonstrated various ester production ability. So, King et al. (2008) noted that all the acetate esters measured were present at higher concentrations in the *Saccharomyces* Vin7/Vin13 coinoculated wines compared with the *Saccharomyces* Vin7/QA23 coinoculated wines indicating that the type of yeast strains used in the coinoculations will affect the volatile composition at the end of fermentation. The same results confirming that cofermentation with two commercial strains can be accompained by chemical profiles that are distinct from those obtained when each strain is fermenting alone were published by Gustafsson et al. (2016). Esters production in Chardonnay wines fermented with six commercial *S. cerevisiae* yeast strains, two individual Burgundian, and four mixed Burgundian *S. cerevisiae* strains were presented in the work by Saberi et al. (2012). The Burgundian yeast strains produced more ethyl hexanoate and ethyl octanoate, and low-to-moderate amounts of the acetate esters compared to industrial strains were tested. In contrast, most of the

3-Methylthiopropan-1-ol	2-Methylpropan-1-ol	1-Propanol	E-3-Hexen-1-ol	Z-3-Hexen-1-ol	Methanol	Refs.
na	13	na	na	na	na	King et al. (2008)
na	14	na	na	na	na	King et al. (2008)
na	13	na	na	na	na	King et al. (2008)
na	15	na	na	na	na	King et al. (2008)

industrial strains produced either very low or very high concentration of acetate esters while in the ethyl ester production no marked differences were determined. Furdíková et al. (2014) examined the influence of selected autochthonous and commercial *S. cerevisiae* strains on the ester production. Main differences were detected in the synthesis of isoamyl acetate whose concentrations were the highest in the commercial *S. cerevisiae* wine while one of the autochthonous strains produced higher concentrations of ethyl lactate and diethyl succinate. Two locally selected strains of *S. cerevisiae* were compared to spontaneous fermentation in the work by Cordero-Bueso et al. (2016). Significant differences detected among tested strains were for isoamyl acetate, hexyl acetate, 2-phenylethyl acetate, and ethyl lactate. During wine fermentation, *S. cerevisiae* is not the only microorganism that can contribute to the aroma and flavor of the wine. Nowadays, great interest has shown for non-*Saccharomyces* species and their role in the production of some novel aromas due to the production of enzymes that are either absent from *S. cerevisiae* or produced in poor quantities. In 2001, Rojas et al. examined *C. dattila*, *H. guilliermondii*, *H. osmophila*, *H. uvarum*, *M. pulcherrima*, *P. anomala*, *P. heedii*, *P. membranaefaciens*, *S. pombe*, *Zygosaccharomyces bailii* yeast strains, and two widely used commercial *S. cerevisiae* strains for the production of acetate esters. Obtained results showed that ethyl acetate was the major component produced by non-*Saccharomyces* yeasts. Also, *H. guilliermondi* was found out to be a potent 2-phenylethyl acetate producer while *P. anomala* produced a significant amount of isoamyl acetate. Further research by Rojas et al. (2003) confirmed the ability of *H. guilliermondi* to produce higher amounts of 2-phenylethyl acetate in pure and mixed culture with *S. cerevisiae* while *P. anomala* strain selected for its ability to synthesize isoamyl acetate produced low levels of this ester. Also, ethyl acetate concentrations produced in wines by *P. anomala* and *H. guilliermondii* pure cultures were greater than those obtained by *S. cerevisiae* yeast. Use of mixed cultures reduced ethyl acetate concentrations, but they were still higher compared to pure *S. cerevisiae* wines. *H. guilliermondii* yeast was the best producer of isobutyl and hexyl acetate while *S. cerevisiae* yeast was the best producer of all ethyl esters. The study reported by Viana et al. (2011) was conducted to evaluate the growth of *H. vineae* as part of a mixed culture with *S. cerevisiae* and its effect on wine volatiles, particularly 2-phenylethyl acetate in simultaneous and sequential inoculation. Achieved results confirmed good *H. vineae* ability to produce 2-phenylethyl acetate, especially in sequential fermentations. Evaluation of *H. vineae* in sequential fermentation with *S. cerevisiae* compared to spontaneous fermentation and pure *S. cerevisiae* strain was also done by Medina et al. (2013). Obtained results pointed out positive influence of *H. vineae* in sequential fermentation in the production of 2-phenylethyl acetate, isobutyl and isoamyl acetate. Also, research by Lleixà et al. (2016) confirmed *H. vineae* ability to synthesize higher concentrations of 2-phenylethyl acetate. Clemente-Jimenez et al. (2005) were studied the use of *P. fermentans* in pure cultures and sequential mixtures with *S. cerevisiae*. *P. fermentans* in sequential mixtures increased the concentrations of ethyl acetate and ethyl caprylate, both qualitatively and quantitatively. Impact of *H. uvarum*, *T. delbrueckii*, and *K. thermotolerans* yeasts alone and together with *S. cerevisiae* mixed, and sequential fermentations were studied by Ciani et al. (2006). Mixed and sequential trials with *T. delbrueckii* and *K. thermotolerans* showed no marked difference in the production of ethyl acetate while *H. uvarum* yeast used alone or together with *S. cerevisiae* caused an unacceptable increase in ethyl acetate. Also in the work by Renault et al. (2009) *T. delbrueckii* produced only small quantities of acetate esters, and ethyl esters of fatty acids. These results are in good agreement with Hernandez-Orte et al. (2008), who describe this species as a low aroma producer compared to other non-*Saccharomyces* wine yeasts, such as the *Hanseniaspora* and *Pichia* genera. *H. guilliermondii* and *H. uvarum* yeasts were investigated in grape must fermentations as pure and mixed starter cultures with *S. cerevisiae* by Moreira et al. (2008). The highest concentrations of 2-phenylethyl acetate were produced when *H. guilliermondii* was inoculated in grape musts, whereas *H. uvarum* raised the isoamyl acetate concentration in wines. Both investigated non-*Saccharomyces* yeasts gave high concentration of ethyl acetate; however, the amount of this compound decleaned in mixed cultures with *S. cerevisiae*. *H. guilliermondii* and *H. uvarum* positively influenced the contents of hexyl acetate while pure or mixed cultures of *S. cerevisiae* give wines with the highest amount of ethyl hexanoate. Higher synthesis of 2-phenylethyl acetate by use of *H. guilliermondii* inoculated fermentation was also confirmed in the work by Moreira et al. (2011). The oenological potential of *H. uvarum* in mixed cultures (simultaneous) and sequential inoculation with *S. cerevisiae* was tasted by Tristezza et al. (2016). Chemical analysis of the wines produced using the mixed cultures of *H. uvarum*/*S. cerevisiae* clearly differ from wine produced with the solo *S. cerevisiae* by higher content of 2-phenylethyl acetate. Viana et al. (2008) used *H. guilliermondii*, *H. osmophila*, and *P. membranifaciens* for enological traits. The lowest and almost similar concentration of ethyl acetate to those observed in wines fermented by commercial *S. cerevisiae* strain was produced by *H. osmophila*. On the contrary the highest amount of that ester was produced by *H. guilliermondii* and *P. membranifaciens* yeasts. The amounts of 2-phenylethyl acetate produced by *H. osmophila* were approximately 10 times greater than those produced by commercial *S. cerevisiae* strains, while *H. guilliermondii* tested strains produced the second largest quantities. No significant differences in isoamyl acetate were found in musts fermented by the non-*Saccharomyce*s yeast strains tested while the highest amount was produced by *S. cerevisiae* yeast. With respect to ethyl esters, no differences were found among yeasts for ethyl caprylate, whereas *H. osmophila* wines showed the highest quantity of ethyl caproate. The levels of ethyl esters produced by non-*Saccharomyces* yeasts were much lower than those detected in *S. cerevisiae* wines what was already shown in the work by Rojas et al. (2003). In the work by Lage et al. (2014) higher amounts of ethyl esters were synthetized by *S. cerevisiae* than by *H. guilliermondii* while the production of 2-phenylethyl acetate was higher in the *H. guilliermondii* fermentations. The influence of mixed cultures of *H. osmophila* and *S. cerevisiae* significantly affected ester concentrations, especially 2-phenylethyl acetate whose concentrations were three to nine times greater compared to pure culture *S. cerevisiae* fermentation. Also the concentrations of ethyl acetate, ethyl caproate, and ethyl caprylate revealed statistically meaningful relation with *H. osmophila* portion in the mixed culture (Viana et al., 2009). Study by Andorrà et al. (2010) examined the effect of mixed *Saccharomyces* and non-*Saccharomyces* cultures on the aroma production in natural grape must. These fermentations were inoculated with a *Saccharomyces* strain together with a *C. zemplinina* strain and/or *H. uvarum* strain. The production of ethyl esters was significantly higher in the presence of non-*Saccharomyces* yeasts and especially in the pure cultures of

C. zemplinina, mostly due to the increase in ethyl octanoate, whereas in the mixed fermentation, it was related to the increases in ethyl lactate. Although all the fermentations produced more acetate esters than the pure *S. cerevisiae* culture, the only significant difference was in the mixed *H. uvarum* and *S. cerevisiae* culture wines mainly due to the higher concentrations of isoamyl, hexyl, and 2-phenylethyl acetate. Results of the analyses of the main volatile compounds as isoamyl acetate, ethyl acetate as well as ethyl esters achieved by Comitini et al. (2011) pointed out that wines produced by *C. zemplinina*/*S. cerevisiae* did not show significant differences compared to pure culture of *S. cerevisiae*. On the contrary, mixed fermentations with *T. delbrueckii* and *L. thermotolerans* at the lower *S. cerevisiae* inoculation levels were characterized by higher productions of the major esters (ethyl acetate and ethyl lactate). *M. pulcherrima* positively influenced the production of isoamyl acetate while mixed fermentation with *T. delbrueckii* resulted with the reduction of isoamyl acetate and 2-phenylethyl acetate. The effect of pure and mixed inocula of *S. cerevisiae*, *H. uvarum*, and *C. zemplinina* on the synthesis of the main acetate and ethyl esters was evaluated by Andorrà et al. (2012). The acetate esters production was more influenced by the presence of non-*Saccharomyces* yeasts. Hexyl acetate was highly produced by single fermentation with *C. zemplinina* as well as in mixed fermentation with all three non-*Saccharomyces* yeasts used. *H. uvarum* fermentations resulted with a higher production of isoamyl acetate while 2-phenylethanol acetate was the only acetate ester highly produced by *S. cerevisiae* as in the presence of non-*Saccharomyces* its concentration was significantly diminished. The amounts of ethyl esters of fatty acids were higher in the mixed-culture fermentations, particularly in the *Candida zemplenina*/*H. uvarum*/*S. cerevisiae* fermentations mainly due to the increase in ethyl lactate and ethyl caprylate production. *C. zemplinina*, *T. delbrueckii*, and *M. pulcherrima* were used to conduct fermentations either in monoculture or in coculture with *S. cerevisiae* in the work by Sadoudi et al. (2012). The positive interaction was observed between *M. pulcherrima* and *S. cerevisiae* what resulted in a higher level of 2-phenylethyl acetate compared to each monoculture fermentations. Interaction between *T. delbrueckii*/*S. cerevisiae* and *C. zemplinina*/*S. cerevisiae* cocultures resulted in the lower levels of 2-phenylethyl acetate compared to *S. cerevisiae* monoculture but higher compared to T. *delbrueckii* and *C. zemplinina* monoculture. In the ethyl ester production the lowest amount was produced by single *C. zemplinina* fermentation while the highest was noted in the *M. pulcherrima*/*S. cerevisiae* wine followed by *C. zemplinina*/*S. cerevisiae* cocultures. Use of *M. pulcherrima* and *Saccharomyces uvarum* in single and sequential fermentation with *S. cerevisiae* was studied by Varela et al. (2016). *M. pulcherrima* yeast produced significantly higher concentrations of ethyl acetate compared to *S. cerevisiae* control wine while all the fermentations where *S. uvarum* was inoculated contained higher 2-phenylethyl acetate concentrations. Comparison of single-strain fermentation of non-*Saccharomyces* yeasts and the autochthonous *S. cerevisiae* by Cordero-Bueso et al. (2013) pointed out some differences in the concentrations of the esters. Significantly the highest amount of acetate esters (2-phenylethyl acetate and isoamyl acetate) was produced by *S. cerevisiae* followed by *T. delbrueckii*, *L. fermentati*, and *H. guilliermondii*. Between them *H. guilliermondii* single-fermentation wine contained the highest concentration of ethyl acetate. The stronger producer of ethyl esters was again *S. cerevisiae* followed by *T. delbrueckii* who singled out on the basis of higher ethyl hexanoate production what was also noted in the work by Sadoudi et al. (2012). Sequential fermentation involving different *T. delbrueckii* strains and compared to pure culture *S. cerevisiae* fermentation was investigated by Loira et al. (2014). Concentrations of some esters like ethyl lactate and 2-phenylethyl acetate were higher in the sequential fermentations even though between them some differences were noted. Azzolini et al. (2012, 2015) investigated the influence of selected and commercially available strains of *T. delbrueckii* in simultaneously and sequentially fermentation with *S. cerevisiae*. The content of isoamyl acetate was found at lower concentrations what was also the case with ethyl esters. In both years ethyl octanoate and ethyl decanoate were detected at lower concentrations in the wines inoculated with *T. delbrueckii* than in the control wines. Impact of *T. delbrueckii* yeast on the ester formation in sequential and mixed cultures with *S. cerevisiae* was studied by Renault et al. (2015). Results confirmed previous findings of low *T. delbrueckii* ability to produce major esters in a single-culture fermentation but also pointed out some esters (ethyl propanoate, ethyl isobutanoate, ethyl dihydrocinnamate) specifically produced by *T. delbrueckii*. In the mixed-culture fermentation enhancement of isoamyl acetate as well as 2-phenylethyl acetate, ethyl butanoate and decanoate was detected while sequential fermentation led to the production of major esters similar to that of *S. cerevisiae*. Study by Rodriguez et al. (2010) evaluated the impact of metabolic interactions between indigenous *S. cerevisiae* and *Candida pulcherrima* strains on alcoholic fermentation behavior and wine aroma. Three inoculation strategies, simultaneous, sequential and final were performed and achieved results confirmed that *C. pulcherrima* in a single-culture fermentation is a week producer of esters compared to *S. cerevisiae*. Sequential inoculation showed the best results as its wines presented the highest ester concentrations mainly due to the higher 2-phenylethyl acetate production. The work by Gobbi et al. (2013) investigated the influence of *L. thermotolerans* and *S. cerevisiae* in simultaneous and sequential fermentation. In microfermentation trail, the notable difference was detected between *L. thermotolerans* in pure culture and other treatments by producing significantly the highest concentration of ethyl lactate. On the contrary, in the industrial trial the wines from the mixed cultures and, in particular, for the sequential culture, showed greatly improved contents of isoamyl acetate, ethyl lactate, ethyl hexanoate, and ethyl butyrate. Balikci et al. (2016) investigated the behavior of *L. thermotolerans* and *S. cerevisiae* in pure, cocultured, and sequential fermentation. Pure culture fermentation with *S. cerevisiae* and simultaneous inoculation of *S. cerevisiae* and *L. thermotolerans* led to higher contents of isoamyl acetate and ethyl acetate compared to pure culture *L. thermotolerans* and sequential fermentation. Similar production patterns were observed for the contents of isobutyl acetate, ethyl butyrate, ethyl hexanoate, and ethyl octanoate in *S. cerevisiae* and simultaneous inoculation wines while sequential fermentations and pure culture fermentation of *L. thermotolerans* were characterized by reduced or no formation of these flavor compounds. Sequential fermentations of *S. cerevisiae* with *P. kluyveri*, *L. thermotolerans*, or *M. pulcherrima* were compared to a single fermentation using *S. cerevisiae* by Benito et al. (2015). Total ethyl esters production, primary ethyl lactate, ethyl butanoate, and ethyl hexanoate were the highest in the wines produced by *L. thermotolerans*/*S. cerevisiae* coinoculation while other two coinoculation combinations were inferior compared to *S. cerevisiae* pure culture fermentation. On the contrary in the 2-phenylethyl acetate production coinoculation by *P. klyveri* showed the best results, followed again by *S. cerevisiae*. Benito et al. (2016a) also evaluated the influence of simultaneous and sequential *L. thermotolerans* and *S. cerevisiae* fermentations. Isoamyl acetate was formed in higher concentrations by pure *S. cerevisiae* and simultaneous fermentation while higher 2-phenylethyl acetate and ethyl lactate were detected in sequential fermentation. In the study by Englezos et al. (2016) *S. bacillaris* (synonym *Candida zemplinina*) and *S. cerevisiae* were evaluated in mixed (coinoculated and sequentially) inoculated fermentations. Mixed-culture fermentations positively influenced production of ethyl esters and hexyl acetate compared to single-culture fermentations while the amount of 2-phenylethyl acetate was similar in wines produced by pure *S. cerevisiae* and mixed-culture yeasts. The effect of sequential inoculation of *W. anomalus* (formerly *Hansenula anomala*) and a commercial *S. cerevisiae* strain was examined by Cañas et al. (2014). Data achieved pointed out positive influence of *W. anomalus* on the concentration of total acetate as well as ethyl esters. In the work by Lombard (2016) influence of *K. aerobia* and *W. anomalus* yeasts in co- and sequential fermentations with two *S. cerevisiae* strains on the aroma profile was investigated. *W. anomalus* produced high concentrations of ethyl acetate and ethyl caproate and caprylate while *K. aerobia* produced higher 2-phenylethyl acetate concentrations. The ability of *K. gamospora*, *L. thermotolerans*, *M. pulcherrima*, *T. delbrueckii*, and *Z. kombuchaensis* in the esters production was evaluated by Whitener et al. (2015). The non-*Saccharomyces* yeasts give lower amounts of esters with the ommision of *K. gamospora* who sythetized more total esters than the *S. cerevisiae* used as a control. *K. gamospora* and *Z. kombuchaensis* strains and their influence on aroma profile was positively evaluated by Dashko et al. (2015). Garavaglia et al. (2015) used *Z. bailii* yeast as a co-starter in fermentations with *S. cerevisiae*. All coinoculated wines contained significantly higher concentrations of ethyl acetate and also ethyl octanoate and ethyl decanoate. Influence of a mixed culture of *D. vanriji* and *S. cerevisiae* showed positive influence on the ester concentration.This was mainly due to the concentration of ethyl lactate, diethyl succinate, ethyl decanoate, ethyl, and isopentyl 4-hydroxybutanoate which can contribute to the fruity flavor of wines (Garcia et al., 2002). In the study by Erten and Tanguler (2010) mixed-culture fermentations of *Williopsis saturnus* and *S. cerevisiae* as well as single-culture fermentation of *S. cerevisiae* as control were conducted. Mixed cultures formed relatively higher amounts of ethyl acetate and isoamyl acetate in comparison with control *S. cerevisiae* fermentation. Study of several selected *S. pombe* strains was performed by Benito et al. (2016b). Between tested strains, no difference was noted in the production of 2-phenylethyl acetate, and isoamyl acetate whose concentrations were lower than the one produced by *S. cerevisiae* strains. On the contrary, two selected *S. pombe* strains singled out by the higher production of ethyl lactate and ethyl acetate, concentrations of the last being significantly higher compared to *S. cerevisiae* strain used (Tables 6.6 and 6.7).

6.4 Conclusion

This chapter presents unified data concerning the influence of different yeast species on the aroma composition of wines. In modern winemaking application of selected strains of *S. cerevisiae* is frequent. There are over 20 yeast genera that could be used in alcohol fermentation. Among them, it is worth to mention *Saccharomycodes*, *Schizosaccharomyces*, *Candida*, *Torulaspora*, *Debaryomyces*, *Issatchenkia*, *Pichia*, *Kluyveromyces*, *Metschnikowia*, *Hanseniaspora (Kloeckera)*, *Brettanomyces (Dekkera)*, and *Zygosaccharomyces*. The abovementioned yeast genera have a great potential to be applied for production of wine with distinct aroma profile. Some non-*Saccharomyces* species such as *T. delbrueckii*, *M. pulcherrima*, and *L. thermotolerans* have a high capacity to release 3MH from its precursor while *M. pulcherrima*, *H. uvarum*, and *L. thermotolerans* are able to produce larger amounts of 4MMP. *Hansenula* sp. strain isolated from fermenting must, *C. molischiana* and *C. wickerhamii* yeast strains have

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
S. cerevisiae	41.71	0.08	0.15	na	0.53	0.84
S. cerevisiae	36.71	0.09	0.19	na	0.67	1.07
T. delbrueckii + *S. cerevisiae*	58.28	0.17	0.29	na	0.60	0.87
T. delbrueckii + *S. cerevisiae*	55.24	0.18	0.33	na	0.68	0.99
S. cerevisiae	na	na	na	11.8	1.3	0.5
T. delbrueckii	na	na	na	27.4	0.7	0.3
K. marxianus	na	na	na	29.2	0.9	0.4
S. pombe	16.32	na	na	6.32	na	na
S. pombe	18.11	na	na	6.58	na	na
S. pombe	18.33	na	na	6.52	na	na
S. pombe	18.62	na	na	6.39	na	na
S. pombe	82.41	na	na	7.76	na	na
S. pombe	89.13	na	na	7.82	na	na
S. cerevisiae	17.45	na	na	7.23	na	na
S. cerevisiae	19.23	na	na	7.54	na	na
S. cerevisiae	17	na	0.49	na	na	na
S. cerevisiae	25.3	na	0.50	na	na	na
S. cerevisiae	22.3	na	0.53	na	na	na
S. cerevisiae	27	na	0.49	na	na	na
S. cerevisiae	54.42	na	na	7.16	0.32	0.36
L. thermotolerans + *S cerevisiae*	53.61	na	na	8.89	0.34	0.39
L. thermotolerans + *S cerevisiae*	50.36	na	na	13.14	0.29	0.25
L. thermotolerans	31.6	na	0.04	na	na	na
S. cerevisiae	46.4	na	0.26	na	0.52	0.28
S. cerevisiae + *L. Thermotolerans*	49.0	na	0.26	na	0.48	0.18
S. cerevisiae + *L. Thermotolerans*	35.3	na	0.10	na	0.08	na
S. cerevisiae + *L. Thermotolerans*	34.7	na	0.05	na	0.04	na
S. cerevisiae + *L. Thermotoleran*	33.0	na	0.05	na	0.05	na

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
0.20	na	na	na	Belda et al. (2017)
0.31	na	na	na	Belda et al. (2017)
0.35	na	na	na	Belda et al. (2017)
0.39	na	na	na	Belda et al. (2017)
0.3	na	na	na	Vigentini et al. (2016)
0.3	na	na	na	Vigentini et al. (2016)
0.1	na	na	na	Vigentini et al. (2016)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Benito et al. (2016a)
na	na	na	na	Gustafsson et al. (2016)
na	na	na	na	Gustafsson et al. (2016)
na	na	na	na	Gustafsson et al. (2016)
na	na	na	na	Gustafsson et al. (2016)
0.11	na	na	na	Benito et al. (2016a)
0.13	na	na	na	Benito et al. (2016a)
0.15	na	na	na	Benito et al. (2016a)
na	na	na	na	Balikci et al. (2016)
na	na	na	na	Balikci et al. (2016)
na	na	na	na	Balikci et al. (2016)
na	na	na	na	Balikci et al. (2016)
na	na	na	na	Balikci et al. (2016)
na	na	na	na	Balikci et al. (2016)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
S. cerevisiae	19.58	na	na	na	0.44	0.44
Z. florentina	12.09	na	na	na	0.03	
Z. florentina + *S. cerevisiae*	10.67	na	na	na	0.36	0.16
Z. florentina + *S. cerevisiae*	8.85	na	na	na	0.22	0.03
Z. florentina + *S. cerevisiae*	7.15	na	na	na	0.18	0.03
H. vineae[a]	na	na	62	8285	81	79
S. cerevisiae[a]	na	na	158	3071	241	225
S. pombe	76.87	na	na	9.78	na	na
S. pombe	74.36	na	na	11.37	na	na
S. pombe	43.19	na	na	9.80	na	na
S. pombe	44.17	na	na	8.83	na	na
S. cerevisiae	63.26	na	na	18.43	na	na
H. uvarum + *S. cerevisiae*[a]	na	na	425	1028	645	548
H. uvarum + *S. cerevisiae*[a]	na	na	319	720	561	573
S. cerevisiae[a]			386	1006	510	406
S. cerevisiae	23.96	0.08	0.05	na	0.11	0.26
M. pulcherrima + *S. cerevisiae*	202.26	0.19	0.106	na	0.16	0.24
S. uvarum + *S. cerevisiae*	19.84	0.31	0.08	na	0.01	0.01
M. pulcherrima + *S. uvarum* + *S. cerevisiae*	23.05	0.39	0.15	na	0.01	0.01
S. cerevisiae	25.53	0.06	0.046	na	0.12	0.16
M. pulcherrima + *S. cerevisiae*	124.85	0.16	0.08	na	0.15	0.18
S. uvarum + *S. cerevisiae*	18.53	0.13	0.13	na	0.02	–

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
0.10	na	na	na	Lencioni et al. (2016)
0.72	na	na	na	Lencioni et al. (2016)
0.15	na	na	na	Lencioni et al. (2016)
0.14	na	na	na	Lencioni et al. (2016)
0.11	na	na	na	Lencioni et al. (2016)
143	na	na	na	Lleixà et al. (2016)
76	na	na	na	Lleixà et al. (2016)
na	na	na	na	Mylona et al. (2016)
na	na	na	na	Mylona et al. (2016)
na	na	na	na	Mylona et al. (2016)
na	na	na	na	Mylona et al. (2016)
na	na	na	na	Mylona et al. (2016)
219	na	na	na	Tristezza et al. (2012)
252	na	na	na	Tristezza et al. (2012)
188	na	na	na	Tristezza et al. (2012)
0.14	na	na	na	Varela et al. (2016)
0.04	na	na	na	Varela et al. (2016)
0.01	na	na	na	Varela et al. (2016)
0.01	na	na	na	Varela et al. (2016)
0.07	na	na	na	Varela et al. (2016)
0.1	na	na	na	Varela et al. (2016)
0.02	na	na	na	Varela et al. (2016)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
M. pulcherrima + *S. uvarum* + *S. cerevisiae*	53.69	0.09	0.17	na	0.17	0.05
S. bayanus	79.4	na	na	na	2.14	3.43
S. cerevisiae	101.4	na	na	na	2.99	4.71
S. cerevisiae	24.4	0.08	0.19	8.01	1.03	1.40
T. delbrueckii + *S. cerevisiae*	24.2	0.10	0.17	6.31	0.79	0.99
M. pulcherrima + *S. cerevisiae*	19.6	–	0.13	6.78	0.68	1.05
T. delbrueckii[a]	na	195	29	na	120	187
T. delbrueckii + *S. cerevisiae* sequental[a]	na	233	220	na	671	580
T. delbrueckii + *S. cerevisiae* simultan[a]	na	110	343	na	1195	1364
S. cerevisiae[a]	na	37	286	na	1301	1407
S. bayanus	75.48	0.07	0.55	9.76	1.21	1.35
K. thermotolerans + *S. bayanus*	70.15	0.07	0.73	14.27	1.4	1.24
P. kluyveri + *S. bayanus*	79.85	0.07	0.57	8.65	1.39	1.53
M. Pulcherrima + *S. bayanus*	57.34	0.06	0.56	8.57	1.32	1.46
S. cerevisiae	34.6	na	0.29	32.3	0.19	0.20
W. anomalus + *S. cerevisiae*	42.9	na	0.24	37.5	0.15	0.16
T. delbrueckii + *S. cerevisiae*	61.7	na	na	17.2	na	na
T. delbrueckii + *S. cerevisiae*	63.7	na	na	9.4	na	na
T. delbrueckii + *S. cerevisiae*	67.5	na	na	6.1	na	na

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
0.04	na	na	na	Varela et al. (2016)
na	na	na	na	Mouret et al. (2015)
na	na	na	na	Mouret et al. (2015)
0.39	na	na	na	González-Royo et al. (2015)
0.17	na	na	na	González-Royo et al. (2015)
0.14	na	na	na	González-Royo et al. (2015)
458	na	na	na	Renault et al. (2009)
533	na	na	na	Renault et al. (2009)
878	na	na	na	Renault et al. (2009)
614	na	na	na	Renault et al. (2009)
0.62	na	na	na	Benito et al. (2015)
0.73	na	na	na	Benito et al. (2015)
0.71	na	na	na	Benito et al. (2015)
0.68	na	na	na	Benito et al. (2015)
0.35	na	na	na	Cañas et al. (2014)
0.27	na	na	na	Cañas et al. (2014)
na	na	na	na	Loira et al. (2014)
na	na	na	na	Loira et al. (2014)
na	na	na	na	Loira et al. (2014)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
T. delbrueckii + *S. cerevisiae*	55.9	na	na	10.4	na	na
T. delbrueckii + *S. cerevisiae*	71.7	na	na	28.3	na	na
S. cerevisiae	54.7	na	na	4.7	na	na
S. uvarum	70.0	na	na	6.5	na	na
S. pombe	24.26	na	na	9.11	na	na
S. pombe + *S. cerevisiae* mix	26.28	na	na	10.36	na	na
S. pombe + *S. cerevisiae* seq	21.14	na	na	11.15	na	na
S. cerevisiae	25.83	na	na	11.10	na	na
S. cerevisiae[a]	na	na	126.1	6598.5	589.1	1048.7
T. delbrueckii1 + *S. cerevisiae*[a]	na	na	83.1	15,816.7	139.6	233.2
T. delbrueckii2 + *S. cerevisiae*[a]	na	na	87.1	8004.2	268.7	317.9
S. cerevisiae[a]	na	na	110.2	3914.9	547.6	836.5
T. delbrueckii1 + *S. cerevisiae*[a]	na	na	80.8	4513.2	195.2	147.2
T. delbrueckii2 + *S. cerevisiae*[a]	na	na	81.6	3193.4	258.8	189.4
S. cerevisiae	41.67	na	0.18	1.29	0.33	0.37
H. guilliermondii + *S. cerevisiae*	165.49	na	0.25	2.84	0.32	0.28
H. guilliermondii	156.78	na	0.03	–	0.02	0.01
S. cerevisiae	na	na	na	155.38	na	9.04
S. cerevisiae	na	na	na	51.34	na	11.27
S. cerevisiae	na	na	na	47.52	na	11.32
S. cerevisiae	na	na	na	17.20	na	8.91
H. vineae + *S. cerevisiae*[a]	14.68	na	na	3186	271	679
S. cerevisiae-spontaneous[a]	5871	na	na	1718	317	1212

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
na	na	na	na	Loira et al. (2014)
na	na	na	na	Loira et al. (2014)
na	na	na	na	Loira et al. (2014)
na	na	na	na	Loira et al. (2014)
na	na	na	na	Benito et al. (2014)
na	na	na	na	Benito et al. (2014)
na	na	na	na	Benito et al. (2014)
na	na	na	na	Benito et al. (2014)
269.1	na	na	na	Azzolini et al. (2015)
79.0	na	na	na	Azzolini et al. (2015)
280.7	na	na	na	Azzolini et al. (2015)
264.3	na	na	na	Azzolini et al. (2015)
77.6	na	na	na	Azzolini et al. (2015)
167.7	na	na	na	Azzolini et al. (2015)
0.44	na	na	na	Lage et al. (2014)
0.44	na	na	na	Lage et al. (2014)
0.02	na	na	na	Lage et al. (2014)
0.84	na	na	na	Furdíková et al. (2014)
1.30	na	na	na	Furdíková et al. (2014)
1.34	na	na	na	Furdíková et al. (2014)
0.87	na	na	na	Furdíková et al. (2014)
685	na	na	na	Medina et al. (2013)
677	na	na	na	Medina et al. (2013)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
S. cerevisiae[a]	8023	na	na	1300	511	1084
S. cerevisiae	19.58	na	na	2.35	0.44	0.44
L. thermotolerans	58.09	na	na	16.69	0.17	0.07
S. cerevisiae + *L. thermotolerans*	43.88	na	na	2.27	0.26	0.24
S. cerevisiae + *L. thermotolerans* seq	47.82	na	na	1.92	0.14	0.06
S. cerevisiae + *L. thermotolerans* seq	54.56	na	na	4.94	0.07	0.06
S. cerevisiae	31.8	na	0.02	na	0.03	0.1
M. pulcherrima + *S. cerevisiae*	207.6	na	0.02	na	0.03	0.05
S. cerevisiae	19.4	na	0.1	na	0.4	0.2
M. pulcherrima + *S. cerevisiae*	53.9	na	0.1	na	0.4	0.1
S. cerevisiae	7.76	na	0.2	na	0.036	0.035
S. cerevisiae	6.95	na	0.27	na	0.045	0.048
S. cerevisiae	5.70	na	0.29	na	0.059	0.059
S. cerevisiae	6.63	na	0.29	na	0.042	0.045
S. cerevisiae	6.24	na	0.36	na	0.03	0.030
S. cerevisiae	8.44	na	0.26	na	0.039	0.036
S. cerevisiae[a]	na	na	6.48	na	850.6	622
M. pulcherrima[a]	na	na	–	na	412.5	88.86
T. delbrueckii[a]	na	na	–	na	720.7	459.6
C. zemplinina[a]	na	na	–	na	44.34	8.91
C. zemplinina + *S. cerevisiae*[a]	na	na	5.52	na	924.6	487.1
M. pulcherrima + *S. cerevisiae*[a]	na	na	6.70	na	1786	864.9
T. delbruecki + *S. cerevisiae*[a]	na	na	3.28	na	857.2	242.8
H. vineae + *S. cerevisiae* simulteneous	81.8	na	na	na	na	0.37
H. vineae + *S. cerevisiae* sequential	138.2	na	na	na	na	0.40

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
560	na	na	na	Medina et al. (2013)
0.10	na	na	na	Gobbi et al. (2013)
0.12	na	na	na	Gobbi et al. (2013)
0.11	na	na	na	Gobbi et al. (2013)
0.11	na	na	na	Gobbi et al. (2013)
0.20	na	na	na	Gobbi et al. (2013)
0.1	na	na	na	Contreras et al. (2014)
0.03	na	na	na	Contreras et al. (2014)
0.1	na	na	na	Contreras et al. (2014)
0.1	na	na	na	Contreras et al. (2014)
na	na	na	na	Saberi et al. (2012)
na	na	na	na	Saberi et al. (2012)
na	na	na	na	Saberi et al. (2012)
na	na	na	na	Saberi et al. (2012)
na	na	na	na	Saberi et al. (2012)
na	na	na	na	Saberi et al. (2012)
41.14	na	na	na	Sadoudi et al. (2012)
22.25	na	na	na	Sadoudi et al. (2012)
43.30	na	na	na	Sadoudi et al. (2012)
–	na	na	na	Sadoudi et al. (2012)
83.62	na	na	na	Sadoudi et al. (2012)
195	na	na	na	Sadoudi et al. (2012)
51.29	na	na	na	Sadoudi et al. (2012)
0.40	na	na	na	Viana et al. (2011)
0.23	na	na	na	Viana et al. (2011)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast —cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
H. osmophila + *S. cerevisiae*	60.94	na	na	12.63	0.11	0.04
P. anomala + *S. cerevisiae*	552.09	na	na	6.52	0.32	0.14
S. ludwigii + *S. cerevisiae*	322.94	na	na	5.06	0.36	0.12
Z. florentinus + *S. cerevisiae*	38.07	na	na	10.92	0.37	0.11
S. cerevisiae	34.34	na	na	8.95	0.20	0.18
S. cerevisiae	85.8	na	0.73	4.44	0.26	0.37
H. guilliermondii	79.1	na	0.78	3.34	0.16	0.20
S. cerevisiae	28.44	na	na	8.15	0.2	0.16
C. zemplinina + *S. cerevisiea*	40.63	na	na	9.99	0.23	0.21
L. thermotolerans + *S. cerevisiae*	40.0	na	na	11.47	0.18	0.13
T. delbrueckii + *S. cerevisiae*	50.52	na	na	6.07	0.22	0.17
M. pulcherrima + *S. cerevisiae*	51.64	na	na	4.16	0.14	0.14
C. pulcherrima	7.09	na	na	na	0.02	na
S. cerevisiae	55.24	na	na	na	0.61	na
C. pulcherrima + *S. cerevisiae* simulaneous	59.51	na	na	na	0.71	na
C. pulcherrima + *S. cerevisiae* sequential	69.41	na	na	na	0.40	na
S. cerevisiae	39.11	na	0.22	na	0.44	0.16
W. saturnus + *S. cerevisiae*	47.89	na	0.25	na	0.52	0.23
W. saturnus + *S. cerevisiae*	43.36	na	0.24	na	0.52	0.27
W. saturnus + *S. cerevisiae*	38.98	na	0.22	na	0.41	0.19

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
0.09	na	na	na	Domizio et al. (2011)
0.18	na	na	na	Domizio et al. (2011)
0.18	na	na	na	Domizio et al. (2011)
0.19	na	na	na	Domizio et al. (2011)
0.30	na	na	na	Domizio et al. (2011)
	na	na	na	Moreira et al. (2008)
	na	na	na	Moreira et al. (2008)
0.40	na	na	na	Comitini et al. (2011)
0.28	na	na	na	Comitini et al. (2011)
0.3	na	na	na	Comitini et al. (2011)
0.31	na	na	na	Comitini et al. (2011)
0.16	na	na	na	Comitini et al. (2011)
na	na	na	na	Rodriguez et al. (2010)
na	na	na	na	Rodriguez et al. (2010)
na	na	na	na	Rodriguez et al. (2010)
na	na	na	na	Rodriguez et al. (2010)
na	na	na	na	Erten and Tanguler (2010)
na	na	na	na	Erten and Tanguler (2010)
na	na	na	na	Erten and Tanguler (2010)
na	na	na	na	Erten and Tanguler (2010)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast —cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
S. cerevisiae	na	na	na	0.34	0.03	0.22
C. zemplina	na	na	na	0.89	0.12	3.6
C. zempina + *S. cerevisiae*	na	na	na	1.27	0.09	0.61
H. uvarum + *S. cerevisiae*	na	na	na	2.27	0.22	0.21
C. zemplina + *H. uvarum* + *S. cerevisiae*	na	na	na	2.54	0.10	0.25
T. delbrueckii	na	na	na	3.12		
S. cerevisiae[a]	61,217.8	87.4	521.1	na	1233.5	1594.1
S. cerevisiae[a]	82,570.1	113.1	408.9	na	1046.9	1441.9
S. cerevisiae[a]	62,768.1	106.7	526.5	na	1378.2	1500.3
S. cerevisiae[a]	80,324.0	93.3	492.9	na	1453.0	2981.7
S. cerevisiae[a]	77,787.0	60.2	715.8	na	1662.4	1880.6
S. cerevisiae[a]	49,303.9	44.9	384.5	na	1251.2	3016.4
S. cerevisiae[a]	64,279.2	99.3	438.0	na	1114.5	1621.0
S. cerevisiae	42.33	na	na	na	0.89	1.26
S. cerevisiae	44.33	na	na	na	0.98	1.20
S. cerevisiae	38.67	na	na	na	0.92	1.37
S. cerevisiae	48.17	na	na	na	1.17	1.38
S. cerevisiae spontaneous	34.67	na	na	na	0.80	1.15
S. cerevisiae	31.3	na	na	na	0.81	na
H. guilliermondii	114.8	na	na	na	0.09	na
H. guilliermondii	228.2	na	na	na	0.06	na
H. osmophila	39.9	na	na	na	0.22	na
P. membranifaciens	292.8	na	na	na	0.04	na
S. cerevisiae spontaneous	136.53	na	0.07	na	0.11	na
S. cerevisiae 1×10^4	79.23	na	0.07	na	0.10	na
S. cerevisiae 1×10^5	62.26	na	0.13	na	0.27	na
S. cerevisiae $1x\ 10^6$	87.04	na	0.08	na	0.14	na

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
na	na	na	na	Andorrà et al. (2010)
na	na	na	na	Andorrà et al. (2010)
na	na	na	na	Andorrà et al. (2010)
na	na	na	na	Andorrà et al. (2010)
na	na	na	na	Andorrà et al. (2010)
na	na	na	na	Renault et al. (2009)
na	na	na	na	Swiegers et al. (2009)
na	na	na	na	Swiegers et al. (2009)
na	na	na	na	Swiegers et al. (2009)
na	na	na	na	Swiegers et al. (2009)
na	na	na	na	Swiegers et al. (2009)
na	na	na	na	Swiegers et al. (2009)
na	na	na	na	Swiegers et al. (2009)
0.44	na	na	na	Torrens et al. (2008)
0.42	na	na	na	Torrens et al. (2008)
0.45	na	na	na	Torrens et al. (2008)
0.47	na	na	na	Torrens et al. (2008)
0.45	na	na	na	Torrens et al. (2008)
0.21	na	na	na	Viana et al. (2008)
0.03	na	na	na	Viana et al. (2008)
0.03	na	na	na	Viana et al. (2008)
0.03	na	na	na	Viana et al. (2008)
0.03	na	na	na	Viana et al. (2008)
na	na	na	na	Furdíková et al. (2014)
na	na	na	na	Furdíková et al. (2014)
na	na	na	na	Furdíková et al. (2014)
na	na	na	na	Furdíková et al. (2014)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast —cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
S. cerevisiae 1×10^7	40.01	na	0.08	na	0.11	na
S. cerevisiae	na	na	na	2.5	0.1	0.4
S. cerevisiae	na	na	na	1.9	0.13	0.5
S. bayanus	na	na	na	2.5	0.12	0.54
H. uvarum	na	na	na	0.66	0.04	0.02
K. apiculata	na	na	na	2.0	0.04	0.05
S. cerevisiae	5.65	na	0.11	na	1.68	16.57
Starm. bacillaris	6.13	na	0.11	na	2.70	17.43
S. cerevisiae + *Starm. bacillaris* coinoculation	6.83	na	0.17	na	3.52	22.39
S. cerevisiae + *Starm. bacillaris* sequential	8.07	na	0.20	na	4.02	21.93
S. cerevisiae	28.05	na	na	na	5.61	29.37
Z. bailii + *S. cerevisae*	144.46	na	na	na	5.61	45.30
Z. bailii + *S. cerevisae*	155.50	na	na	na	5.98	44.55
Z. baili + *S. cerevisae*	193.71	na	na	na	6.58	45.26
Z. bailii	133.74	na	na	na	14.57	1693.86
S. cerevisiae[a]	13,943	204	1060	5003	721	458
H. uvarum + *S. cerevisiae*[a]	16,474	288	1111	25,460	816	755
H. uvarum + *S. cerevisiae*[a]	51,706	408	892	37,072	550	319
S. cerevisiae spontaneous	68.71	na	na	–	0.60	1.01
S. cerevisiae	69.28	na	na	2.24	0.52	1.10
S. cerevisiae	64.65	na	na	–	0.39	0.73
S. cerevisiae	81.1	0.11	0.21	na	0.69	1.34
S. cerevisiae	54.8	0.03	0.26	na	0.96	1.42
S. cerevisiae	90.0	0.05	0.14	na	0.48	1.01
S. cerevisiae	55.7	0.06	0.12	na	0.48	1.00
S. cerevisiae	74.0	0.04	0.24	na	0.82	1.21
S. cerevisiae	10.0	0.05	0.22	na	0.80	1.44

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
na	na	na	na	Furdíková et al. (2014)
1.10	na	na	na	Gil et al. (1996)
0.63	na	na	na	Gil et al. (1996)
2.3	na	na	na	Gil et al. (1996)
0.06	na	na	na	Gil et al. (1996)
0.15	na	na	na	Gil et al. (1996)
18.46	na	na	na	Englezos et al. (2016)
18.05	na	na	na	Englezos et al. (2016)
21.98	na	na	na	Englezos et al. (2016)
20.83	na	na	na	Englezos et al. (2016)
63.14	na	na	na	Garavaglia et al. (2015)
145.28	na	na	na	Garavaglia et al. (2015)
157.12	na	na	na	Garavaglia et al. (2015)
126.83	na	na	na	Garavaglia et al. (2015)
377.25	na	na	na	Garavaglia et al. (2015)
65	na	na	na	Hu et al. (2018)
129	na	na	na	Hu et al. (2018)
45	na	na	na	Hu et al. (2018)
0.08	na	na	na	Cordero-Bueso et al. (2016)
0.23	na	na	na	Cordero-Bueso et al. (2016)
–	na	na	na	Cordero-Bueso et al. (2016)
0.53	na	na	na	Srisamatthakarn (2011)
0.44	na	na	na	Srisamatthakarn (2011)
0.42	na	na	na	Srisamatthakarn (2011)
0.46	na	na	na	Srisamatthakarn (2011)
0.40	na	na	na	Srisamatthakarn (2011)
0.56	na	na	na	Srisamatthakarn (2011)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast —cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
Saccharomyces spp.	53.3	0.03	0.22	na	0.97	1.44
Saccharomyces spp.	83.0	0.03	0.24	na	0.98	1.59
S. cerevisiae	–	0.03	0.25	na	0.94	1.43
S. cerevisiae	79.3	0.03	0.27	na	0.95	1.44
S. cerevisiae	–	0.02	0.22	na	0.98	1.42
S. cerevisiae	64.1	0.05	0.18	na	0.65	1.26
S. cerevisiae	88.3	0.06	0.21	na	0.76	1.27
W. anomalus	394.10	na	na	na	na	0.13
W. anomalus	562.02	na	na	na	na	0.12
K. aerobia	319.81	na	na	na	na	0.07
K. aerobia	312.46	na	na	na	na	0.12
W. anomalus + *S. cerevisiae*	210.78	na	na	na	na	0.20
W. anomalus + *S. cerevisiae*	305.17	na	na	na	na	0.21
K. aerobia + *S. cerevisiae*	184.20	na	na	na	na	0.18
K. aerobia + *S. cerevisiae*	176.76	na	na	na	na	0.19
S. cerevisiae	74.56	na	na	na	na	0.33
S. cerevisiae	na	na	na	14.94	0.78	0.78
C. zemplinina	na	na	na	9.77	0.82	2.96
C. zemplinina + *S. cerevisiae*	na	na	na	16.31	1.02	1.56
H. uvarum + *S. cerevisiae*	na	na	na	16.27	1.13	1.36
C. zemplinina + *H. uvarum* + *S. cerevisiae*	na	na	na	23.79	1.21	0.98
S. pombe + *S. cerevisiae*-mixed	na	na	na	na	0.83	0.56
S. pombe + *S. cerevisiae* mixed	na	na	na	na	0.84	0.59

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
0.43	na	na	na	Srisamatthakarn (2011)
0.51	na	na	na	Srisamatthakarn (2011)
0.51	na	na	na	Srisamatthakarn (2011)
0.43	na	na	na	Srisamatthakarn (2011)
0.47	na	na	na	Srisamatthakarn (2011)
0.50	na	na	na	Srisamatthakarn (2011)
0.40	na	na	na	Srisamatthakarn (2011)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
na	na	na	na	Lombard (2016)
0.5	na	na	na	Andorrà et al. (2012)
–	na	na	na	Andorrà et al. (2012)
1.45	na	na	na	Andorrà et al. (2012)
0.83	na	na	na	Andorrà et al. (2012)
0.81	na	na	na	Andorrà et al. (2012)
na	4.76	0.60	na	Loira et al. (2014)
na	5.03	0.59	na	Loira et al. (2014)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast —cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
S. pombe + *S. cerevisiae* 7VA mixed	na	na	na	na	0.90	0.60
T. delbrueckii + *S. cerevisiae* mixed	na	na	na	na	1.11	0.73
T. delbrueckii + *S. cerevisiae* mixed	na	na	na	na	1.19	0.65
T. delbrueckii + *S. cerevisiae* mixed	na	na	na	na	1.02	0.55
S. pombe + *S. cerevisiae*-seq	na	na	na	na	1.72	0.37
S. pombe + *S. cerevisiae* seq	na	na	na	na	0.83	0.47
S. pombe + *S. cerevisiae* seq	na	na	na	na	0.68	0.47
T. delbrueckii + *S. cerevisiae* seq	na	na	na	na	1.12	0.39
T. delbrueckii + *S. cerevisiae* seq	na	na	na	na	1.07	0.59
T. delbrueckii + *S. cerevisiae* seq	na	na	na	na	0.81	0.35
S. cerevisiae	na	na	na	na	1.23	0.76
S. uvarum	na	na	na	na	1.88	0.69
S. cerevisiae	39.5	na	na	0.33	–	0.60
C. stellata	53.9	na	na	0.09	0.01	0.01
H. guilliermondii	874.4	na	na	0.04	–	0.05
L. fermentati	50.2	na	na	0.10	0.04	0.13
S. cerevisiae	na	na	na	na	na	na
T. delbrueckii	na	na	na	na	na	na
K. marxianus	na	na	na	na	na	na
H. vineae[a]	na	na	na	na	na	na
S. cerevisiae[a]	na	na	na	na	na	na
H. uvarum + *S. cerevisiae*[a]	na	na	na	na	na	na

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
na	5.07	0.52	na	Loira et al. (2014)
na	5.11	0.63	na	Loira et al. (2014)
na	6.20	0.61	na	Loira et al. (2014)
na	5.03	0.55	na	Loira et al. (2014)
na	0.43	0.15	na	Loira et al. (2014)
na	0.58	0.12	na	Loira et al. (2014)
na	0.66	0.13	na	Loira et al. (2014)
na	5.11	0.14	na	Loira et al. (2014)
na	9.54	0.17	na	Loira et al. (2014)
na	5.84	0.12	na	Loira et al. (2014)
na	6.86	0.74	na	Loira et al. (2014)
na	4.03	0.32	na	Loira et al. (2014)
na	na	na	na	Cordero-Bueso et al. (2013)
na	na	0.09	0.03	Cordero-Bueso et al. (2013)
na	na	0.18	0.08	Cordero-Bueso et al. (2013)
na	na	0.14	0.13	Cordero-Bueso et al. (2013)
na	0.9	2.3	na	Viana et al. (2011)
na	0.3	1.5	na	Viana et al. (2011)
na	0.3	1.1	na	Viana et al. (2011)
na	119	na	1240	Lleixà et al. (2016)
na	52	na	1775	Lleixà et al. (2016)
na	52	na	5648	Tristezza et al. (2016)

Continued

Table 6.6 Dependence of Ethyl-Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast —cont'd

Yeast	Ethyl Acetate	Ethyl Propanoate	Ethyl Butanoate	Ethyl Lactate	Ethyl Hexanoate	Ethyl Octanoate
H. uvarum + *S. cerevisiae*[a]	na	na	na	na	na	na
S. cerevisiae[a]	na	na	na	na	na	na
S. cerevisiae[a]	na	na	na	na	na	na
W. anomalus + *S. cervisiae*[a]	na	na	na	na	na	na
S. cerevisiae[a]	na	na	na	na	na	na
T. delbruecki1 + *S. cerevisiae*[a]	na	na	na	na	na	na
T. delbruecki2 + *S. cerevisiae*[a]	na	na	na	na	na	na
S. cerevisiae[a]	na	na	na	na	na	na
T. delbruecki1 + *S. cerevisiae*[a]	na	na	na	na	na	na
T. delbruecki2 + *S. cerevisiae*[a]	na	na	na	na	na	na
H. vineae + *S. cerevisiae*[a]	na	na	na	na	na	na
S. cerevisiae-spontaneous[a]	na	na	na	na	na	na
S. cerevisiae[a]	na	na	na	na	na	na
T. delbrueckii	na	na	na	na	na	na
S. cerevisiae	na	na	na	na	na	na
S. cerevisiae	na	na	na	na	na	na
S. bayanus	na	na	na	na	na	na
H. uvarum	na	na	na	na	na	na
K. apiculata	na	na	na	na	na	na

na, not analyzed.
Results are expressed in mg/L except other is not indicate.
[a] Results are expressed in μg/L.

Ethyl Decanoate	Ethyl-3-Hydroxybutanoate	Ethyl-4-Hydoxybutanoate	Ethyl Succinate	Refs.
na	65	na	6052	Tristezza et al. (2016)
na	52	na	8476	Tristezza et al. (2016)
na	79.6	2.08	na	Cañas et al. (2014)
na	56	1.96	na	Cañas et al. (2014)
na	314.1	na	na	Azzolini et al. (2015)
na	288.3	na	na	Azzolini et al. (2015)
na	229.2	na	na	Azzolini et al. (2015)
na	289.7	na	na	Azzolini et al. (2015)
na	131.1	na	na	Azzolini et al. (2015)
na	224.1	na	na	Azzolini et al. (2015)
na	172	6216	na	Medina et al. (2013)
na	129	14.40	na	Medina et al. (2013)
na	126	12.43	na	Medina et al. (2013)
na	0.06	na	na	Renault et al. (2009)
na	0.15	na	na	Gil et al. (1996)
na	0.2	na	na	Gil et al. (1996)
na	0.28	na	na	Gil et al. (1996)
na	0.03	na	na	Gil et al. (1996)
na	0.04	na	na	Gil et al. (1996)

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
S. cerevisiae	0.02	1.33	0.07	na	0.21	na	na	na	Belda et al. (2017)
S. cerevisiae	na	1.42	0.06	na	0.21	na	na	na	Belda et al. (2017)
T. delbrueckii + *S. cerevisiae*	0.04	2.28	0.10	na	1.11	na	na	na	Belda et al. (2017)
T. delbrueckii + *S. cerevisiae*	0.03	1.86	0.07	na	1.06	na	na	na	Belda et al. (2017)
S. cerevisiae	na	0.8	na	0.9	0.01	na	na	na	Vigentini et al. (2016)
T. delbrueckii	na	0.2	na	0.6	0.01	na	na	na	Vigentini et al. (2016)
K. marxianus	na	0.5	na	0.8	0.06	na	na	na	Vigentini et al. (2016)
S. pombe	na	2.28	na	na	5.25	na	na	na	Benito et al. (2016a)
S. pombe	na	2.21	na	na	5.36	na	na	na	Benito et al. (2016a)
S. pombe	na	2.44	na	na	5.29	na	na	na	Benito et al. (2016a)
S. pombe	na	2.33	na	na	5.38	na	na	na	Benito et al. (2016a)
S. pombe	na	2.68	na	na	5.48	na	na	na	Benito et al. (2016a)
S. pombe	na	2.59	na	na	5.54	na	na	na	Benito et al. (2016a)

S. cerevisiae	na	3.77	na	na	6.41	na	na	na	Benito et al. (2016a)
S. cerevisiae	na	3.62	na	na	6.34	na	na	na	Benito et al. (2016a)
S. cerevisiae	na	1.4	na	na	na	na	na	na	Gustafsson et al. (2016)
S. cerevisiae	na	1.5	na	na	na	na	na	na	Gustafsson et al. (2016)
S. cerevisiae	na	1.6	na	na	na	na	na	na	Gustafsson et al. (2016)
S. cerevisiae	na	1.5	na	na	na	na	na	na	Gustafsson et al. (2016)
S. cerevisiae	na	–	na	na	0.36	na	na	na	Benito et al. (2016a)
L. thermotolerans + *S. cerevisiae*	na	–	na	na	0.39	na	na	na	Benito et al. (2016a)
L. thermotolerans + *S. cerevisiae*	na	–	na	na	0.52	na	na	na	Benito et al. (2016a)
L. thermotolerans	na	0.15	na	na	na	na	na	na	Balikci et al. (2016)
S. cerevisiae	na	5.94	0.14	na	na	na	na	na	Balikci et al. (2016)
S. cerevisiae + *L. Thermotolerans*	na	6.0	0.16	na	na	na	na	na	Balikci et al. (2016)
S. cerevisiae + *L. Thermotolerans*	na	0.32	na	na	na	na	na	na	Balikci et al. (2016)
S. cerevisiae + *L. Thermotolerans*	na	0.18	na	na	na	na	na	na	Balikci et al. (2016)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
S. cerevisiae + *L. Thermotoleran*	na	0.17	na	na	na	na	na	na	Balikci et al. (2016)
S. cerevisiae	na	0.77	na	na	0.11	na	na	na	Lencioni et al. (2016)
Z. florentina	na	0.01	na	na	0.78	na	na	na	Lencioni et al. (2016)
Z. florentina + *S. cerevisiae*	na	0.46	na	na	0.54	na	na	na	Lencioni et al. (2016)
Z. florentina + *S. cerevisiae*	na	0.27	na	na	0.98	na	na	na	Lencioni et al. (2016)
Z. florentina + *S. cerevisiae*	na	0.19	na	na	0.77	na	na	na	Lencioni et al. (2016)
H. vineae[a]	9	222	na	na	2322	na	na	na	Lleixà et al. (2016)
S. cerevisiae[a]	14	218	na	na	47	na	na	na	Lleixà et al. (2016)
S. pombe	na	3.72	na	na	8.50	na	na	na	Mylona et al. (2016)
S. pombe	na	13.63	na	na	8.02	na	na	na	Mylona et al. (2016)
S. pombe	na	7.66	na	na	7.11	na	na	na	Mylona et al. (2016)
S. pombe	na	7.42	na	na	6.98	na	na	na	Mylona et al. (2016)
S. cerevisiae	na	10.81	na	na	7.80	na	na	na	Mylona et al. (2016)

H. uvarum + *S. cerevisiae*[a]	na	2535	na	na	598	na	na	na	Tristezza et al. (2012)
H. uvarum + *S. cerevisiae*[a]	na	2239	na	na	696	na	na	na	Tristezza et al. (2012)
S. cerevisiae[a]	na	2235	na	na	517	na	na	na	Tristezza et al. (2012)
S. cerevisiae	na	0.70	0.22	na	0.26	na	na	na	Varela et al. (2016)
M. pulcherrima + *S. cerevisiae*	na	0.42	0.13	na	0.20	na	na	na	Varela et al. (2016)
S. uvarum + *S. cerevisiae*	na	0.13	0.05	na	0.47	na	na	na	Varela et al. (2016)
M. pulcherrima + *S. uvarum* + *S. cerevisiae*	na	0.15	0.06	na	0.53	na	na	na	Varela et al. (2016)
S. cerevisiae	na	0.18	0.06	na	0.09	na	na	na	Varela et al. (2016)
M. pulcherrima + *S. cerevisiae*	na	0.22	0.07	na	0.23	na	na	na	Varela et al. (2016)
S. uvarum + *S. cerevisiae*	na	0.09	0.03	na	0.66	na	na	na	Varela et al. (2016)
M. pulcherrima + *S. uvarum* + *S. cerevisiae*	na	0.10	0.03	na	0.63	na	na	na	Varela et al. (2016)
S. bayanus	na	6.66	na	na	4.91	na	na	na	Mouret et al. (2015)
S. cerevisiae	na	14.06	na	na	12.24	na	na	na	Mouret et al. (2015)
S. cerevisiae	na	0.46	na	na	2.37	na	na	na	González-Royo et al. (2015)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
T. delbrueckii + *S. cerevisiae*	na	0.62	na	na	1.78	na	na	na	González-Royo et al. (2015)
M. pulcherrima + *S. cerevisiae*	na	0.53	na	na	1.82	na	na	na	González-Royo et al. (2015)
T. delbrueckii[a]	na	64	na	na	53	na	na	na	Renault et al. (2009)
T. delbrueckii + *S. cerevisiae* sequental[a]	na	3834	na	na	455	na	na	na	Renault et al. (2009)
T. delbrueckii + *S. cerevisiae* simultaneous[a]	na	4957	na	na	540	na	na	na	Renault et al. (2009)
S. cerevisiae[a]	na	1828	na	na	435	na	na	na	Renault et al. (2009)
S. bayanus	na	3.66	na	na	0.95	na	na	na	Benito et al. (2015)
K. thermotolerans + *S. bayanus*	na	3.33	na	na	0.54	na	na	na	Benito et al. (2015)
P. kluyveri + *S. bayanus*	na	3.04	na	na	1.23	na	na	na	Benito et al. (2015)

M. Pulcherrima + *S. bayanus*	na	2.44	na	na	0.82	na	na	na	Benito et al. (2015)
S. cerevisiae	na	1.64	na	na	0.58	na	na	na	Cañas et al. (2014)
W. anomalus + *S. cerevisiae*	na	1.89	na	na	0.67	na	na	na	Cañas et al. (2014)
T. delbrueckii + *S. cerevisiae*	na	3.5	na	na	19.1	na	na	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae*	na	5.0	na	na	33.2	na	na	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae*	na	5.5	na	na	18.3	na	na	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae*	na	3.6	na	na	30.2	na	na	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae*	na	3.4	na	na	28.7	na	na	na	Loira et al. (2014)
S. cerevisiae	na	4.1	na	na	15.2	na	na	na	Loira et al. (2014)
S. uvarum	na	4.3	na	na	11.9	na	na	na	Loira et al. (2014)
S. pombe	na	1.93	na	na	3.49	na	na	na	Benito et al. (2014)
S. pombe + *S. cerevisiae* mix	na	7.18	na	na	5.34	na	na	na	Benito et al. (2014)
S. pombe + *S. cerevisiae* seq	na	4.45	na	na	3.51	na	na	na	Benito et al. (2014)
S. cerevisiae	na	15.14	na	na	5.59	na	na	na	Benito et al. (2014)
S. cerevisiae[a]	na	1869.8	na	na	357.5	na	na	na	Azzolini et al. (2015)
T. delbrueckii1 + *S. cerevisiae*[a]	na	988.4	na	na	487.8	na	na	na	Azzolini et al. (2015)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
T. delbruecki2 + *S. cerevisiae*[a]	na	639.6	na	na	339.5	na	na	na	Azzolini et al. (2015)
S. cerevisiae[a]	na	1338.7	na	na	265.9	na	na	na	Azzolini et al. (2015)
T. delbruecki1 + *S. cerevisiae*[a]	na	748.2	na	na	207.7	na	na	na	Azzolini et al. (2015)
T. delbruecki2 + *S. cerevisiae*[a]	na	624.2	na	na	212.2	na	na	na	Azzolini et al. (2015)
S. cerevisiae	na	3.92	na	na	1.33	na	na	na	Lage et al. (2014)
H. guilliermondii + *S. cerevisiae*	na	4.06	na	na	5.63	na	na	na	Lage et al. (2014)
H. guilliermondii	na	0.33	na	na	5.44	na	na	na	Lage et al. (2014)
S. cerevisiae	na	0.62	na	na	na	na	na	na	Furdíková et al. (2014)
S. cerevisiae	na	0.74	na	na	na	na	na	na	Furdíková et al. (2014)
S. cerevisiae	na	0.51	na	na	na	na	na	na	Furdíková et al. (2014)
S. cerevisiae	na	1.59	na	na	na	na	na	na	Furdíková et al. (2014)

H. vineae + *S. cerevisiae*[a]	na	948	na	na	4341	na	na	na	Medina et al. (2013)
S. cerevisiae-spont.[a]	na	685	na	na	756	na	na	na	Medina et al. (2013)
S. cerevisiae[a]	na	871	na	na	425	na	na	na	Medina et al. (2013)
S. cerevisiae	na	0.77	na	na	0.11	na	na	na	Gobbi et al. (2013)
L. thermotolerans	na	0.18	na	na	0.02	na	na	na	Gobbi et al. (2013)
S. cerevisiae + *L. thermotolerans*	na	0.39	na	na	0.02	na	na	na	Gobbi et al. (2013)
S. cerevisiae + *L. thermotolerans* seq	na	0.24	na	na	0.01	na	na	na	Gobbi et al. (2013)
S. cerevisiae + *L. thermotolerans* seq	na	0.33	na	na	0.02	na	na	na	Gobbi et al. (2013)
S. cerevisiae	na	7.8	na	na	na	na	na	na	Contreras et al. (2014)
M. pulcherrima + *S. cerevisiae*	na	15.9	na	na	na	na	na	na	Contreras et al. (2014)
S. cerevisiae	na	7.0	na	na	na	na	n.a	na	Contreras et al. (2014)
M. pulcherrima + *S. cerevisiae*	na	6.1	na	na	na	na	n.a	na	Contreras et al. (2014)
S. cerevisiae	na	0.09	na	na	0.028	na	na	na	Saberi et al. (2012)
S. cerevisiae	na	0.1	na	na	0.031	na	na	na	Saberi et al. (2012)
S. cerevisiae	na	0.05	na	na	0.034	na	na	na	Saberi et al. (2012)
S. cerevisiae	na	0.11	na	na	0.025	na	na	na	Saberi et al. (2012)
S. cerevisiae	na	0.1	na	na	0.024	na	na	na	Saberi et al. (2012)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
S. cerevisiae	na	0.22	na	na	0.022	na	na	na	Saberi et al. (2012)
S. cerevisiae[a]	na	3669	na	na	871.7	na	na	na	Sadoudi et al. (2012)
M. pulcherrima[a]	na	92.41	na	na	170.2	na	na	na	Sadoudi et al. (2012)
T. delbrueckii[a]	na	496.6	na	na	202.7	na	na	na	Sadoudi et al. (2012)
C. zemplinina[a]	na	45.62	na	na	21	na	na	na	Sadoudi et al. (2012)
C. zemplinina + *S. cerevisiae*[a]	na	671.7	na	na	542.4	na	na	na	Sadoudi et al. (2012)
M. pulcherrima + *S. cerevisiae*[a]	na	4736	na	na	2212	na	na	na	Sadoudi et al. (2012)
T. delbruecki + *S. cerevisiae*[a]	na	878	na	na	574.3	na	na	na	Sadoudi et al. (2012)
H. vineae + *S. cerevisiae* simulteneous	na	5.83	na	na	0.81	na	na	na	Viana et al. (2011)
H. vineae + *S. cerevisiae* sequential	na	4.09	na	na	1.70	na	na	na	Viana et al. (2011)
H. osmophila + *S. cerevisiae*	na	0.28	na	na	0.24	na	na	na	Domizio et al. (2011)

P. anomala + *S. cerevisiae*	na	0.65	na	na	0.22	na	na	na	Domizio et al. (2011)
S. ludwigii + *S. cerevisiae*	na	0.75	na	na	0.11	na	na	na	Domizio et al. (2011)
Z. florentinus + *S. cerevisiae*	na	0.38	na	na	0.63	na	na	na	Domizio et al. (2011)
S. cerevisiae	na	0.26	na	na	0.30	na	na	na	Domizio et al. (2011)
S. cerevisiae	na		na	na	0.35	na	na	na	Moreira et al. (2008)
H. guilliermondii	na		na	na	0.73	na	na	na	Moreira et al. (2008)
S. cerevisiae	na	0.26	na	na	na	na	na	na	Comitini et al. (2011)
C. zemplinina + *S. cerevisiea*	na	0.15	na	na	na	na	na	na	Comitini et al. (2011)
L. thermotolerans + *S. cerevisiae*	na	0.19	na	na	na	na	n.a	na	Comitini et al. (2011)
T. delbrueckii + *S. cerevisiae*	na	0.42	na	na	na	na	n.a	na	Comitini et al. (2011)
M. pulcherrima + *S. cerevisiae*	na	0.98	na	na	na	na	n.a	na	Comitini et al. (2011)
C. pulcherrima	na	0.02	na	na	0.02	na	na	na	Rodriguez et al. (2010)
S. cerevisiae	na	4.95	na	na	0.11	na	na	na	Rodriguez et al. (2010)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
C. pulcherrima + *S. cerevisiae* simulaneous	na	4.87	na	na	0.13	na	na	na	Rodriguez et al. (2010)
C. pulcherrima + *S. cerevisiae* sequential	na	5.93	na	na	0.33	na	na	na	Rodriguez et al. (2010)
S. cerevisiae	na	6.10	na	na	na	na	na	na	Erten and Tanguler (2010)
W. saturnus + *S. cerevisiae*	na	7.42	na	na	na	na	na	na	Erten and Tanguler (2010)
W. saturnus + *S. cerevisiae*	na	6.94	na	na	na	na	na	na	Erten and Tanguler (2010)
W. saturnus + *S. cerevisiae*	na	6.19	na	na	na	na	na	na	Erten and Tanguler (2010)
S. cerevisiae	na	0.25	na	na	2.99	na	na	na	Andorrà et al. (2010)
C. zemplina	na	0.15	na	na	1.09	na	na	na	Andorrà et al. (2010)
C. zempina + *S. cerevisiae*	na	0.14	na	na	2.79	na	na	na	Andorrà et al. (2010)
H. uvarum + *S. cerevisiae*	na	0.70	na	na	4.23	na	na	na	Andorrà et al. (2010)
C. zemplina + *H. uvarum* + *S. cerevisiae*	na	0.2	na	na	3.31	na	na	na	Andorrà et al. (2010)

T. delbrueckii	na	59.94	na	na	0.89	na	na	na	Renault et al. (2009)
S. cerevisiae[a]	na	na	242.6	na	359.0	na	na	na	Swiegers et al. (2009)
S. cerevisiae[a]	na	na	398.6	na	835.2	na	na	na	Swiegers et al. (2009)
S. cerevisiae[a]	na	na	236.0	na	459.9	na	na	na	Swiegers et al. (2009)
S. cerevisiae[a]	na	na	394.8	na	589.8	na	na	na	Swiegers et al. (2009)
S. cerevisiae[a]	na	na	315.9	na	553.5	na	na	na	Swiegers et al. (2009)
S. cerevisiae[a]	na	na	152.3	na	460.2	na	na	na	Swiegers et al. (2009)
S. cerevisiae[a]	na	na	236.0	na	292.0	na	na	na	Swiegers et al. (2009)
S. cerevisiae	na	3.48	na	na	0.21	na	na	na	Torrens et al. (2008)
S. cerevisiae	na	3.56	na	na	0.24	na	na	na	Torrens et al. (2008)
S. cerevisiae	na	4.01	na	na	0.27	na	na	na	Torrens et al. (2008)
S. cerevisiae	na	3.42	na	na	0.25	na	na	na	Torrens et al. (2008)
S. cerevisiae spontaneous	na	2.90	na	na	0.20	na	na	na	Torrens et al. (2008)
S. cerevisiae	na	1.5	na	na	0.05	na	na	na	Viana et al. (2008)
H. guilliermondii	na	0.33	na	na	1.55	na	na	na	Viana et al. (2008)
H. guilliermondii	na	0.39	na	na	2.98	na	na	na	Viana et al. (2008)
H. osmophila	na	0.37	na	na	12.9	na	na	na	Viana et al. (2008)
P. membranifaciens	na	0.28	na	na	0.18	na	na	na	Viana et al. (2008)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
S. cerevisiae spontaneous	na	0.61	na	na	n.a	na	na	na	Furdíková et al. (2014)
S. cerevisiae 1×10^4	na	0.77	na	na	n.a	na	na	na	Furdíková et al. (2014)
S. cerevisiae 1×10^5	na	1.59	na	na	n.a	na	na	na	Furdíková et al. (2014)
S. cerevisiae 1×10^6	na	1.05	na	na	n.a	na	na	na	Furdíková et al. (2014)
S. cerevisiae 1×10^7	na	0.78	na	na	n.a	na	na	na	Furdíková et al. (2014)
S. cerevisiae	na	na	na	n.a	0.1	na	na	na	Gil et al. (1996)
S. cerevisiae	na	na	na	n.a	0.12	na	na	na	Gil et al. (1996)
S. bayanus	na	na	na	n.a	0.24	na	na	na	Gil et al. (1996)
H. uvarum	na	na	na	n.a	0.04	na	na	na	Gil et al. (1996)
K. apiculata	na	na	na	n.a	0.08	na	na	na	Gil et al. (1996)
S. cerevisiae	na	na	na	n.a	1.12	na	na	na	Englezos et al. (2016)
Starm. bacillaris	na	na	na	n.a	0.51	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* coinoculation	na	na	na	n.a	1.11	na	na	na	Englezos et al. (2016)
S. cerevisiae + *Starm. bacillaris* sequential	na	na	na	n.a	1.39	na	na	na	Englezos et al. (2016)

S. cerevisiae	na	na	na	n.a	na	na	na	na	Garavaglia et al. (2015)
Z. bailii + *S. cerevisae*	na	na	na	n.a	na	na	na	na	Garavaglia et al. (2015)
Z. bailii + *S. cerevisae*	na	na	n.a	na	na	n.a	na	na	Garavaglia et al. (2015)
Z. bailii + *S. cerevisae*	na	na	n.a	na	na	n.a	na	na	Garavaglia et al. (2015)
Z. bailii	na	na	n.a	na	na	n.a	na	na	Garavaglia et al. (2015)
S. cerevisiae[a]	na	759	na	na	48	na	na	na	Hu et al. (2018)
H. uvarum + *S. cerevisiae*[a]	na	819	na	na	81	na	na	na	Hu et al. (2018)
H. uvarum + *S. cerevisiae*[a]	na	1285	na	na	139	na	na	na	Hu et al. (2018)
S. cerevisiae spontaneous	na	7.66	na	na	0.56	na	na	na	Cordero-Bueso et al. (2016)
S. cerevisiae	na	9.37	na	na	0.56	na	na	na	Cordero-Bueso et al. (2016)
S. cerevisiae	na	6.19	na	na	0.38	na	na	na	Cordero-Bueso et al. (2016)
S. cerevisiae	na	na	29.3	na	0.10	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	19.6	na	0.06	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	18.0	na	0.10	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	14.1	na	0.06	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	28.3	na	0.09	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	49.1	na	0.12	na	na	na	Srisamatthakarn (2011)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
Saccharomyces spp.	na	na	21.5	na	0.07	na	na	na	Srisamatthakarn (2011)
Saccharomyces spp.	na	na	21.9	na	0.07	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	20.2	na	0.07	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	21.9	na	0.07	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	17.7	na	0.07	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	29.8	na	0.10	na	na	na	Srisamatthakarn (2011)
S. cerevisiae	na	na	22.7		0.09	na	na	na	Srisamatthakarn (2011)
W. anomalus	na	1.95	na	na	0.68	na	na	na	Lombard (2016)
W. anomalus	na	2.31	na	na	0.72	na	na	na	Lombard (2016)
K. aerobia	na	1.37	na	na	1.25	na	na	na	Lombard (2016)
K. aerobia	na	1.31	na	na	1.40	na	na	na	Lombard (2016)
W. anomalus + *S. cerevisiae*	na	2.33	na	na	0.61	na	na	na	Lombard (2016)
W. anomalus + *S. cerevisiae*	na	1.94	na	na	0.65	na	na	na	Lombard (2016)

K. aerobia + *S. cerevisiae*	na	1.69	na	na	1.08	na	na	na	Lombard (2016)
K. aerobia + *S. cerevisiae*	na	1.83	na	na	1.17	na	na	na	Lombard (2016)
S. cerevisiae	na	2.04	na	na	0.53	na	na	na	Lombard (2016)
S. cerevisiae	na	3.11	na	na	5.06	na	na	na	Andorrà et al. (2012)
C. zemplinina	na	3.26	na	na	1.52	na	na	na	Andorrà et al. (2012)
C. zemplinina + *S. cerevisiae*	na	4.14	na	na	3.42	na	na	na	Andorrà et al. (2012)
H. uvarum + *S. cerevisiae*	na	5.09	na	na	3.42	na	na	na	Andorrà et al. (2012)
C. zemplinina + *H. uvarum* + *S. cerevisiae*	na	5.25	na	na	1.29	na	na	na	Andorrà et al. (2012)
S. pombe + *S. cerevisiae*-mixed	na	8.33	na	0.25	na	na	n.a	na	Loira et al. (2014)
S. pombe + *S. cerevisiae* mixed	na	9.01	na	0.29	na	na	n.a	na	Loira et al. (2014)
S. pombe + *S. cerevisiae* mixed	na	8.13	na	0.22	na	na	n.a	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae* mixed	na	11.21	na	0.39	na	na	n.a	na	Loira et al. (2014)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
T. delbrueckii + *S. cerevisiae* mixed	na	11.18	na	0.37	na	na	n.a	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae* mixed	na	11.79	na	0.40	na	na	n.a	na	Loira et al. (2014)
S. pombe + *S. cerevisiae*-seq	na	na	na	na	na	na	n.a	na	Loira et al. (2014)
S. pombe + *S. cerevisiae* seq	na	0.08	na	na	na	na	n.a	na	Loira et al. (2014)
S. pombe + *S. cerevisiae* seq	na	0.1	na	na	na	na	n.a	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae* seq	na	5.99	na	0.18	na	na	na	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae* seq	na	9.59	na	0.28	na	na	na	na	Loira et al. (2014)
T. delbrueckii + *S. cerevisiae* seq	na	3.63	na	0.16	na	na	na	na	Loira et al. (2014)
S. cerevisiae	na	9.96	na	0.42	na	na	na	na	Loira et al. (2014)
S. uvarum	na	5.91	na	0.23	na	na	na	na	Loira et al. (2014)
S. cerevisiae	na	7.66	na	0.14	0.08	na	na	na	Cordero-Bueso et al. (2013)

C. stellata	na	0.01	na	0.01	0.03	na	na	na	Cordero-Bueso et al. (2013)
H. guilliermondii	na	0.08	na	–	0.08	na	na	na	Cordero-Bueso et al. (2013)
L. fermentati	na	0.07	na	0.04	0.13	na	na	na	Cordero-Bueso et al. (2013)
L. thermotolerans	na	0.03	na	–	0.06	na	na	na	Cordero-Bueso et al. (2013)
P. kudriavzevii	na	0.02	na	0.04	0.02	na	na	na	Cordero-Bueso et al. (2013)
P. membranifaciens	na	0.01	na	–	0.03	na	na	na	Cordero-Bueso et al. (2013)
S. pombe	na	0.02	na	0.03	0.16	na	na	na	Cordero-Bueso et al. (2013)
T. delbrueckii	na	1.37	na	0.12	0.19	na	na	na	Cordero-Bueso et al. (2013)
W. anomalus	na	0.04	na	0.01	0.01	na	na	na	Cordero-Bueso et al. (2013)
S. cerevisiae/S. kudriavzev	na	4.5	0.23	0.58	0.89	na	na	na	King et al. (2008)
S. cerevisiae	na	8.6	0.38	0.86	1.1	na	na	na	King et al. (2008)
S. cerevisiae	na	9.3	0.70	0.79	1.4	na	na	na	King et al. (2008)
S. cerevisiae Vin7/QA23-coinoculation	na	5.1	0.26	0.76	1.2	na	na	na	King et al. (2008)
S. cerevisiae Vin7/Vin13-coinoculation	na	8.8	0.60	0.80	1.8	na	na	na	King et al. (2008)
S. cerevisiae	na	na	na	n.a	na	na	na	na	Viana et al. (2011)
T. delbrueckii	na	na	na	n.a	na	na	na	na	Viana et al. (2011)
K. marxianus	na	na	na	na	na	na	na	na	Viana et al. (2011)
H. vineae[a]	na	na	na	na	na	11	88	4012	Lleixà et al. (2016)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
S. cerevisiae[a]	na	na	na	na	na	na	428	15,671	Lleixà et al. (2016)
H. uvarum + *S. cerevisiae*[a]	na	na	na	na	na	na	340	3735	Tristezza et al. (2016)
H. uvarum + *S. cerevisiae*[a]	na	na	na	na	na	na	525	4216	Tristezza et al. (2016)
S. cerevisiae[a]	na	na	na	na	na	na	291	3851	Tristezza et al. (2016)
S. cerevisiae	na	na	na	na	na	0.03	na	na	Varela et al. (2016)
M. pulcherrima + *S. cerevisiae*	na	na	na	na	na	0.09	na	na	Varela et al. (2016)
S. uvarum + *S. cerevisiae*	na	na	na	na	na	na	na	na	Varela et al. (2016)
M. pulcherrima + *S. uvarum* + *S. cerevisiae*	na	na	na	na	na	na	na	na	Varela et al. (2016)
S. cerevisiae	na	na	na	na	na	0.03	na	na	Varela et al. (2016)
M. pulcherrima + *S. cerevisiae*	na	na	na	na	na	0.05	na	na	Varela et al. (2016)
S. uvarum + *S. cerevisiae*	na	na	na	na	na	0.01	na	na	Varela et al. (2016)

M. pulcherrima + *S. uvarum* + *S. cerevisiae*	na	na	na	na	na	0.01	na	na	Varela et al. (2016)
S. bayanus	na	na	na	na	na	0.16	na	na	Mouret et al. (2015)
S. cerevisiae	na	na	na	na	na	0.40	na	na	Mouret et al. (2015)
S. cerevisiae	na	na	na	na	na	0.02	na	0.41	González-Royo et al. (2015)
T. delbrueckii + *S. cerevisiae*	na	na	na	na	na	0.02	na	0.48	González-Royo et al. (2015)
M. pulcherrima + *S. cerevisiae*	na	na	na	na	na	0.02	na	0.53	González-Royo et al. (2015)
S. cerevisiae[a]	na	na	na	na	na	na	20.4	130	Cañas et al. (2014)
W. anomalus + *S. cervisiae*[a]	na	na	na	na	na	na	19.6	110	Cañas et al. (2014)
S. cerevisiae[a]	na	na	na	na	na	na	280.9	369.0	Azzolini et al. (2015)
T. delbruecki1 + *S. cerevisiae*[a]	na	na	na	na	na	na	29.5	4649.6	Azzolini et al. (2015)
T. delbruecki2 + *S. cerevisiae*[a]	na	na	na	na	na	na	40.3	846.2	Azzolini et al. (2015)
S. cerevisiae[a]	na	na	na	na	na	na	1053.3	545.7	Azzolini et al. (2015)
T. delbruecki1 + *S. cerevisiae*[a]	na	na	na	na	na	na	903.1	852.2	Azzolini et al. (2015)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
T. delbruecki2 + *S. cerevisiae*[a]	na	na	na	na	na	na	606.2	579.6	Azzolini et al. (2015)
S. cerevisiae	na	na	na	na	na	na	na	103.35	Furdíková et al. (2014)
S. cerevisiae	na	na	na	na	na	na	na	62.98	Furdíková et al. (2014)
S. cerevisiae	na	na	na	na	na	na	na	64.96	Furdíková et al. (2014)
S. cerevisiae	na	na	na	na	na	na	na	85.10	Furdíková et al. (2014)
H. vineae + *S. cerevisiae*[a]	na	na	na	na	na	na	na	653	Medina et al. (2013)
S. cerevisiae-spont.[a]	na	na	na	na	na	na	na	684	Medina et al. (2013)
S. cerevisiae[a]	na	na	na	na	na	na	na	678	Medina et al. (2013)
W. saturnus + *S. cerevisiae*	na	na	na	na	na	0.14	na	na	Erten and Tanguler (2010)
W. saturnus + *S. cerevisiae*	na	na	na	na	na	0.16	na	na	Erten and Tanguler (2010)
W. saturnus + *S. cerevisiae*	na	na	na	na	na	0.15	na	na	Erten and Tanguler (2010)
T. delbrueckii	na	na	na	na	na		na	0.02	Renault et al. (2009)

S. cerevisiae	na	na	na	na	na	0.064	na	na	Torrens et al. (2008)
S. cerevisiae	na	na	na	na	na	0.059	na	na	Torrens et al. (2008)
S. cerevisiae	na	na	na	na	na	0.066	na	na	Torrens et al. (2008)
S. cerevisiae	na	na	na	na	na	0.058	na	na	Torrens et al. (2008)
S. cerevisiae spontaneous	na	na	na	na	na	0.065	na	na	Torrens et al. (2008)
S. cerevisiae	na	na	na	na	na	0.03	na	0.51	Viana et al. (2008)
H. guilliermondii	na	na	na	na	na	0.012	na	1.64	Viana et al. (2008)
H. guilliermondii	na	na	na	na	na	0.026	na	0.93	Viana et al. (2008)
H. osmophila	na	na	na	na	na	–	na	2.54	Viana et al. (2008)
P. membranifaciens	na	na	na	na	na	0.023	na	0.84	Viana et al. (2008)
S. cerevisiae	na	na	na	na	na	0.28	na	1.78	Gil et al. (1996)
S. cerevisiae	na	na	na	na	na	0.27	na	1.68	Gil et al. (1996)
S. bayanus	na	na	na	na	na	0.32	na	5.1	Gil et al. (1996)
H. uvarum	na	na	na	na	na	0.54	na	0.15	Gil et al. (1996)
K. apiculata	na	na	na	na	na	0.70	na	0.17	Gil et al. (1996)
S. cerevisiae	na	na	na	na	na	na	na	0.11	Englezos et al. (2016)
Starm. bacillaris	na	na	na	na	na	na	na	0.05	Englezos et al. (2016)
S. cerevisiae + Starm. bacillaris coinoculation	na	na	na	na	na	na	na	0.06	Englezos et al. (2016)

Continued

Table 6.7 Dependence of Different Esters Concentration Upon *Saccharomyces* and Non-*Saccharomices* Yeast—cont'd

Yeast	Methyl Butanoate	Isoamyl Acetate	2-Methylbutyl Acetate	Diethyl Butanedioate	2-Phenylethyl Acetate	Isobutyl Acetate	Diethyl Malate	Diethyl Succinate	Refs.
S. cerevisiae + *Starm. bacillaris* sequential	na	na	na	na	na	na	na	0.08	Englezos et al. (2016)
S. cerevisiae[a]	na	na	na	na	na	98	na	na	Hu et al. (2018)
H. uvarum + *S. cerevisiae*[a]	na	na	na	na	na	96	na	na	Hu et al. (2018)
H. uvarum + *S. cerevisiae*[a]	na	na	na	na	na	134	na	na	Hu et al. (2018)
S. cerevisiae spontaneous	na	na	na	na	na	na	na	0.03	Cordero-Bueso et al. (2016)
S. cerevisiae	na	na	na	na	na	na	na	0.09	Cordero-Bueso et al. (2016)
S. cerevisiae	na	na	na	na	na	na	na	0.05	Cordero-Bueso et al. (2016)

na, not analyzed.
Results are expressed in mg/L except other is not indicate.
[a] Results are expressed in μg/L.

a high activity of β-glycosidase thus these species can release bound forms of volatile compounds contained in the grape berries. Despite the ability to relese aroma compounds, most non-*Saccharomyces* species are not able to consume all available sugar present in must so they must be applied as a part of sequential inoculation, mixed, or multistarter cultures with *S. cerevisiae* strains.

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7

NUTRITIONAL COMPONENTS OF SOME FERMENTED NONALCOHOLIC BEVERAGES

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7.1 Introduction

Throughout human history, food fermentation has been the most common method used for protecting perishable foods. As a result of this, the nutritional values of these foods have been maintained and in some cases even improved (Holzapfel, 2015). Fermentation is usually a desirable process for biochemical modification of primary food products, resulting from microorganisms and their enzyme activities. It increases the possibility of improving the food characteristics such as taste, flavor, texture and nutritional value as well as shelf life (Gotcheva et al., 2000).

Fermented foods were accepted to be inherently healthy. The mechanism behind this protection was clarified in 1857 after Louis Pasteur identified "lactic yeast" as the source of lactic acid fermentation. A first "scientific" presentation of fermented foods as health products came in the early 1900s. Ilya Metchnikoff advertized yogurt that was fermented with the Bulgarian bacillus, and insisted that it could contribute to longevity (Metchnikoff, 1907; Holzapfel, 2015). In the 1930s, Minoru Shirota isolated a health-promoting microorganism and introduced the oldest and still-existing probiotic food, Yakult (Holzapfel, 2015).

Fermented foods provide health benefits to the consumer who consumes them. Some of the potential health benefits of fermented foods that have been determined in the recent past were based on an extensive body of anecdotal information, and contain such benefits such as blood glucose lowering effects (Kamiya et al., 2013; Oh et al., 2014; Frias et al., 2017), antihypertensive activity (Ferreira et al., 2006; Nakamura et al., 2013; Koyama et al., 2014; Ahrén et al., 2015;

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Frias et al., 2017), antithrombotic (Kamiya et al., 2013; Frias et al., 2017), and antidiarrheal properties (Parvez et al., 2006; Kamiya et al., 2013; Frias et al., 2017). This is associated with bioactive nutritive molecules (nutrients, vitamins, minerals, fibers, etc.) as well as nonnutritive phytochemicals (phenolic compounds, flavonoids, bioactive peptides, etc.) contents of fermented foods. The products like fermented beverages are part of various diets worldwide. The fermentation of lactic acid bacteria is common among the diverse fermentation processes used. It causes changes in both profile and types of bioactive compounds. While sugar content or antinutritional compounds are decreased, molecules such as bioactive peptides, short-chain fatty acids, and polysaccharides are generated and phenolic compounds are converted to molecules with enhanced biological value. These transformations related to prebiotic and/or probiotic potential availability as well as improvement of food components bioaccessibility and bioavailability ensue positive changes of health-related properties (Septembre-Malaterre et al., 2018).

While the nutritional composition of product changes with fermentation; its health effects vary on parallel. Health benefits of fermented beverages are due to its enriched nutritional composition. These two phenomena are interconnected. In this chapter, manufacturing technologies, composition and nutritive values, health-promoting effects of some fermented beverages are described.

7.2 Shalgam Juice

Shalgam (also spelled Shalgam or Şalgam) juice is a traditional beverage which is mostly produced in some provinces of Southern Turkey, especially in Adana, Mersin, Hatay, and Kahramanmaras in homes, villages, and industries (Canbas and Fenercioglu, 1984; Canbas and Deryaoglu, 1993; Tanguler and Erten, 2012b; Baser et al., 2012b). Shalgam is widely consumed with meals and as a refreshing beverage mainly in these provinces. Also, it is a popular drink in metropolitan cities such as Istanbul, Ankara, and Izmir Metropolis (Canbas and Fenercioglu, 1984; Canbas and Deryaoglu, 1993; Tanguler and Erten, 2012b).

Salgam juice is known as a nonalcoholic fermented beverage. It is produced from turnips (*Brassica rapa*), black or purple carrots (*Daucus carota*), sourdough, bulgur (broken wheat) flour, salt, and water (Turker et al., 2004; Baschali et al., 2017). Kanji is a product similar to shalgam juice, which is produced in India. It is made via the natural fermentation of carrots and by the addition of salt, chilies, and crushed mustard. Both products owe their color to the anthocyanins existed in the black carrot (Altay et al., 2013; Baschali et al., 2017).

7.2.1 Manufacturing Technology of Shalgam Juice

Shalgam juice is generally produced at a home-scale level; on the other hand, small quantities are being commercially produced (Erten et al., 2008; Baschali et al., 2017). There is no standard manufacturing method for shalgam production and processing is different from one plant to another. There are two basic production methods for commercial production, the traditional method and the direct method (Erten et al., 2008; Tanguler and Erten, 2012b). The traditional method consists of two different fermentation stages: sourdough fermentation (first fermentation), performed for the enrichment of lactic acid bacteria and yeast, and carrot fermentation [second (main) fermentation] (Erginkaya and Hammes, 1992; Erten et al., 2008; Tanguler and Erten, 2012b; Altay et al., 2013; Kristbergsson and Ötles, 2016). But, the direct method consist of only second fermentation (Erginkaya and Hammes, 1992; Erten et al., 2008; Altay et al., 2013).

In the traditional method, sourdough fermentation is made by adding salt, sourdough, and water to bulgur flour. Sourdough is obtained from baker's yeast dough which is fermented for a few hours or overnight at room temperature (Erten et al., 2008; Altay et al., 2013). Then, the mixture of bulgur flour, salt, sourdough, and water is left to ferment for 3–5 days at an ambient temperature (Tanguler and Erten, 2012b; Kristbergsson and Ötles, 2016). As a result of sourdough fermentation, the number of lactic acid bacteria and yeast increase (Tanguler and Erten, 2012b; Altay et al., 2013). After the first fermentation, the mixture is extracted with water and then, salt, sliced black carrot, and sliced turnip are added to the extract in a tank for carrot fermentation (second fermentation). The obtained mixture is fermented for 3–10 days depending upon the temperature (Canbas and Fenercioglu, 1984; Erten et al., 2008; Altay et al., 2013).

In the direct method consisting only the second fermentation stage, the chopped black carrots, if available sliced turnip, salt, bakers' yeast (*Saccharomyces cerevisiae*) or sourdough, and adequate water are left to ferment at ambient temperature (Erten et al., 2008; Kristbergsson and Ötles, 2016).

At the end of the second fermentation in the commercial production techniques, a red colored, cloudy, and sour shalgam juice is obtained by filtration. It is packaged in sealed bottles and plastic containers (Fig. 7.1; Canbas and Fenercioglu, 1984; Erten et al., 2008; Altay et al., 2013).

Generally it is accepted that the main fermentation agents of shalgam are lactic acid bacteria. They are responsible for the acidification process by converting sugars into mainly lactic acid and other end compounds that give the typical taste and flavor to shalgam (Erten et al., 2008; Tanguler and Erten, 2012b). Lactic acid bacteria can be

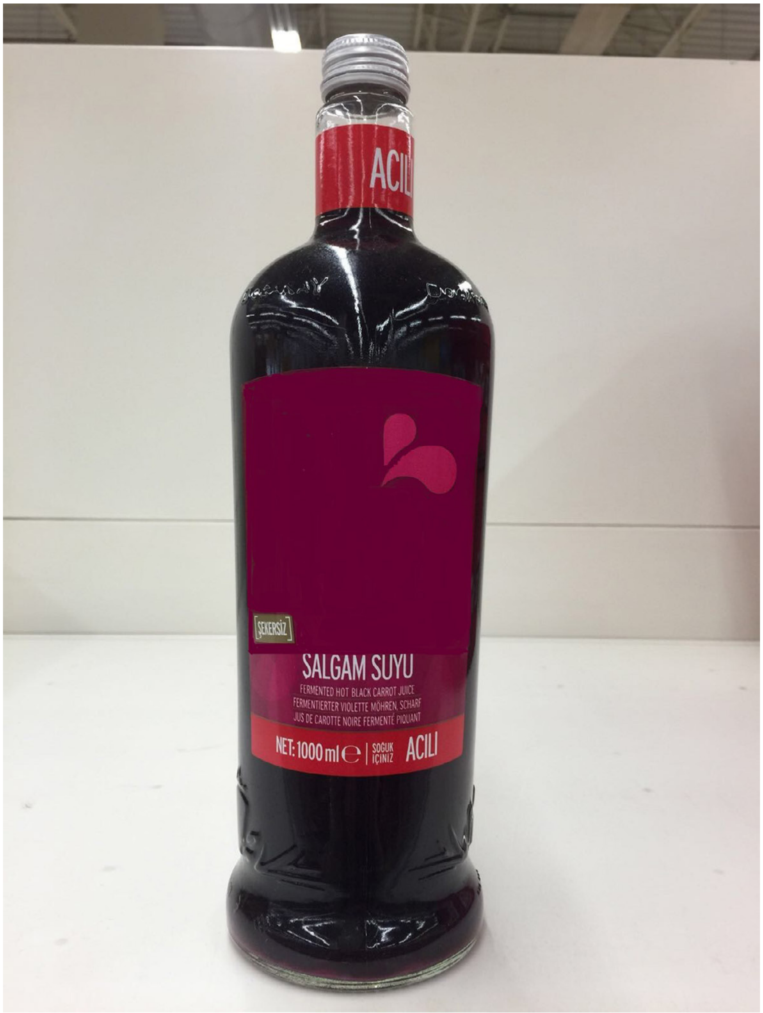
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Fig. 7.1 Shalgam juice.

separated into two groups: homofermentative and heterofermentative. Homofermentative lactic acid bacteria such as *Lactobacillus* (*L.*), *Pediococcus* (*P.*), *Lactococcus* (*Lac.*), and *Streptococcus* form lactic acid that is the major or sole metabolic end product of glucose fermentation using Embden-Meyerhof-Parnas pathway. Heterofermentative lactic acid bacteria such as *Leuconostocs* (*Leu.*), *Oenococcus*, *Weissella* and some *Lactobacillus* produce the equimolar amounts of lactate, CO_2, and acetate or ethanol depending on air by hexose monophosphate or pentose pathway (Holzapfel and Wood, 1995; Caplice and Fitzgerald, 1999; Blandino et al., 2003; Tanguler and Erten, 2012b). The production and consumption of shalgam are increasing in Turkey. Its quality is closely related to the microbial ecology, especially lactic acid bacteria, of fermentation. As is known, no commercial lactic acid bacteria starter cultures are isolated from their natural environments for use in the shalgam fermentations. Shalgam is produced with uncontrolled fermentation and this type fermentation leads to variations with regard to quality and stability of the product. For this reason, it is important to select the starter cultures for the controlled fermentations in order to produce a better product. However, shalgam microbiology is quite complex and cannot be known in detail (Erten et al., 2008; Tanguler and Erten, 2012b). Characterization of dominant microbial populations in commercially available bottled shalgam juice was performed by Baser et al. (2012a). The predominant species in shalgam juice are *Lactobacillus* species including *Lactobacillus casei* ATCC 334, *Lactobacillus casei*, *Lactobacillus casei* subsp. *casei*, *Lactobacillus plantarum* ATCC 14917, *Lactobacillus plantarum* subsp. plantarum ST-III chromosome, *Lactobacillus plantarum* JDM1, *Lactobacillus plantarum* subsp., *Lactobacillus plantarum* subsp. *argentoratensis*, *Lactobacillus acidophilus*, *Lactobacillus brevis* ATCC 367, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Lactobacillus helveticus* DSM 20075, *Lactobacillus helveticus* DPC 4571, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus paracasei* subsp. *tolerans*, *Lactobacillus parabrevis*, *Lactobacillus reuteri*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *delbrueckii*, *Lactobacillus delbrueckii* subsp. *indicus*, *Lactobacillus gasseri*, *Lactobacillus sharpeae*. The shalgam juice appears to be a potential source of beneficial lactic acid bacteria (Baser et al., 2012a). It also contains *Saccharomyces* and non-*Saccharomyces* yeasts. Yeasts which come in with the sourdough play a role in fermentation stages. Although the level of *Saccharomyces* and non-*Saccharomyces* yeasts is lower than the level of lactic acid bacteria, their contribution to fermentation has not been explained (Tanguler and Erten, 2012b; Altay et al., 2013).

7.2.2 Composition and Nutritive Value of Shalgam Juice

The composition of shalgam juice as reported by several authors are total dry matter (2.0%–4.0%), ash (1.46%–2.06%), protein (0.09%–0.018%), lactic acid (0.578–8.05 g/L), salt (1.1%–2.2%), and ethanol (0.79%–6.41 g/L). The titratable acidity is 1.06–9.1 g/L, the amount of CO_2 is 0.44–0.79 g/L, the color index (D520) is 71–131, and the pH varies between 3.15 and 4.25 (Canbas and Fenercioglu, 1984; Canbas and Deryaoglu, 1993; Arici, 2004; Erten et al., 2008; Özdestan and Üren, 2010; Tanguler and Erten, 2012a; Altay et al., 2013;

Kristbergsson and Ötles, 2016). The presence of sugar is not expected in the shalgam juice because of the fermentation (Erten et al., 2008; Altay et al., 2013).

Shalgam juice is a nutritional drink owing to rich mineral, vitamin, amino acid, and polyphenol contents and high antioxidant capacity (Erten et al., 2008; Incedayi et al., 2008; Altay et al., 2013; Okcu et al., 2016). In the shalgam juice, major elements (sodium, potassium, calcium, magnesium, and phosphorus) and minor elements (iron, manganese, tin, copper, nickel, zinc, lead, and cadmium) are present. The elements present in highest amount are calcium (34.02–148.30 mg/L), magnesium (30.61–75.38 mg/L), and phosphorus (8.72–82.96 mg/L). The concentrations of heavy metals including tin, nickel, lead, and cadmium found in shalgam beverage are below 1 mg/L in general (Yilmaz-Ersan and Turan, 2012). Shalgam juice includes A, C, and B group vitamins especially thiamin and riboflavin (Kristbergsson and Ötles, 2016; Baschali et al., 2017). The predominant phenolic compound in shalgam juice is anthocyanins. Black carrot anthocyanins, which consist of cyanidins' derivatives including a high ratio of monoacylated anthocyanins, are responsible for the purplish red color of shalgam juice (Turker et al., 2007; Kristbergsson and Ötles, 2016). It has been expressed that the content of anthocyanin as cyanidin-3-glycoside is between 88.3 and 114.1 mg/L (Canbaş, 1991; Altay et al., 2013). Generally, the amount of anthocyanin contained in homemade shalgam juice is higher (83.19 mg/L) than commercial products (41.89–48.40 mg/L) (Ercelebi and Özkanli, 2010; Altay et al., 2013). It has been reported that the antioxidant capacities of shalgam juice are between 3.53 and 5.93 μmol TE/mL in DPPH, 91–3.61 μmol TE/mL in FRAP, and 2.43–3.96 μmol TE/mL in ABTS assays (Baser et al., 2012b).

7.2.3 Health Benefits of Shalgam Juice Consumption

Studies on the effects of shalgam juice on human health are very limited. When these studies were examined, it has been seen that shalgam juice has positive health benefits like decrease in the coronary heart disease risks. It has anticarcinogenic properties (Incedayi et al., 2008; Kristbergsson and Ötles, 2016). Ozcan et al. (2012) reported that shalgam juice significantly inhibited the growth of Caco-2 colorectal adenocarcinoma cells in vitro. Shalgam juice has also been known to have a positive effect on the digestive system due to its probiotic effects and to behave as an appetizer (Turker et al., 2007; Kristbergsson and Ötles, 2016).

7.3 Boza

Boza is a nonalcoholic beverage produced from the fermentation of cereals such as barley, oats, millet, maize, wheat, and rice (Botes et al., 2007). According to related national regulations, beverages that contain ethyl alcohol <5.0 g/L are accepted as nonalcoholic beverages and alcohol content of boza is between 0.03% and 0.39% (w/v) (Anonymous, 1998; Kose and Yucel, 2003; Yeğin and Üren, 2008). Therefore, boza is considered as a drink that locates in group of nonalcoholic beverages.

There are restricted products manufactured by fermented cereals for the consumption of human. Boza is one of the well-known cereal-based fermented beverages (Arici and Daglioglu, 2002; Heperkan et al., 2014). The origin of boza can be traced back to 8000–9000 years ago and it is believed to date back to the ancient populations that lived in pre-Ottoman in Turkey (Arici and Daglioglu, 2002; Kristbergsson and Ötles, 2016; Baschali et al., 2017). The Ottomans spread the recipe of boza over the countries they conquered. Moreover, the Ottoman Empire was known to feed their army with boza because of its rich nutritional value (Kristbergsson and Ötles, 2016).

The "boza" word comes from the "buze" Persian word, which means millet (Leatherman and Wilster, 1944; Baschali et al., 2017). Boza is widely drunk in Turkey and in Balkan Peninsula including Bulgaria (Sofia, Varna, Burgas), Albania, Romania, and South Russia. While "braga" or "brascha" is a similar beverage consumed in East European countries, "Busa" is another similar beverage consumed in the Balkans (cocoa is added in the standard boza recipe). "Bouza" is also a similar beverage consumed in Egypt (Arici and Daglioglu, 2002; Baschali et al., 2017). Other boza-like products are South African kaffir beer, Kenyan busaa, Nigerian ogi, skete, and pito (Hayta et al., 2001; Yeğin and Üren, 2008).

In Bulgaria, boza is consumed all year round, mainly at breakfast, whereas Turkish people serve it with cinnamon and roasted chickpeas and it is enjoyed mainly during the winter months. The color of boza is light to dark beige and its structure is viscous. It has a sweet to sour bread-like taste (Gotcheva et al., 2000, 2001). It can be classified as sweet or sour boza depending on the acid content. The taste of boza is related with the ingredients used and the method of preparation. The refreshing effect of lactic acid provides boza to be consumed during summer. But, the high temperatures during that season ensue in rapid growth of indigenous microflora and unwanted changes in sensory properties of boza occur (Akpinar-Bayizit et al., 2010; Hui, 2012). For this reason, the consumption of boza is mostly preferred in winter. The popularity of this beverage is due to its flavor, pleasant taste, and nutritional properties (Hui, 2012).

7.3.1 Manufacturing Technology of Boza

Boza can be manufactured both at an artisanal and industrial scale (Hui, 2012). The production of Boza involves six stages (Fig. 7.2; Arici and Daglioglu, 2002; Altay et al., 2013).

Two or more cereals are usually washed and milled to the size of semolina (300–800 lm). They are cooked in a steam boiler after adding water (Kabak and Dobson, 2011; Osimani et al., 2015). The ratio of cereal and water is about 1:5. The cooking process is completed as soon as the raw materials become thick soup-like consistency. After the slurry is cooled and distributed to the containers, they are subjected to fermentation process which takes 1–2 days at 20–22°C. Boza can be consumed by children and elderly from all age groups without any treatment (Heperkan et al., 2014).

For the industrial production of boza, one volume of the cereal mixture is mixed with 4–6 volumes of water under continuous stirring (Arici and Daglioglu, 2002; Hui, 2012). The cereals are cooked in

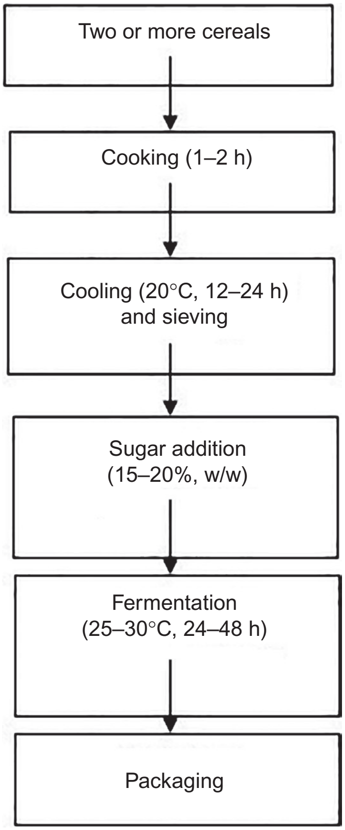
Two or more cereals
Cooking (1–2 h)
Cooling (20°C, 12–24 h) and sieving
Sugar addition (15–20%, w/w)
Fermentation (25–30°C, 24–48 h)
Packaging

Fig. 7.2 Stages of boza production.

an autoclave at 4–5 atmospheres for about 1–2 h, depending on the boiling temperature and the raw material used (Arici and Daglioglu, 2002; Blandino et al., 2003; Hui, 2012; Osimani et al., 2015). Hot water is added several times until the end of the process because of the fact that the mixture absorbs water. The cooking process is stopped as soon as a homogeneous mash is obtained (Arici and Daglioglu, 2002; Hui, 2012). Then, the mixture is cooled to 20°C in the ~12–24 h. Cold water is added and they are sieved for the elimination of undesirable particles (Arici, 2004; Hui, 2012; Osimani et al., 2015). Sugar or saccharine (15%–20%, w/w) are added to the mixture (Kabak and Dobson, 2011; Mendez-Vilas, 2011; Osimani et al., 2015). Some of the added sugar is utilized as a substrate by lactic acid bacteria and yeast (Hui, 2012). The obtained mixture is fermented at 25–30°C for 24–48 h by adding boza obtained from a previous production as a starter culture. If the boza is not available, sourdough or yogurt can be used as a starter culture (at 2%–3%, v/v) (Mendez-Vilas, 2011; Kabak and Dobson, 2011; Caputo et al., 2012; Hui, 2012; Osimani et al., 2015).

Boza fermentation includes two different types of fermentation. First, the alcoholic fermentation occurs. Carbon dioxide bubbles are produced and the volume increases. The lactic acid fermentation then produces in lactic acid which gives the acidic character to boza. The wooden barrels should not be fully filled because of the increase in volume during fermentation. The fermentation rate is reduced by cold storage for the purpose of increasing the shelf life of boza in practical (Arici and Daglioglu, 2002; Holzapfel, 2015). Therefore, fermented boza is cooled to +4°C and packaged in plastic bottles. It is stored under refrigerator conditions (Kabak and Dobson, 2011; Mendez-Vilas, 2011; Caputo et al., 2012; Osimani et al., 2015). It is recommended that boza be consumed within a few days after production to prevent an excessive sour taste. In the first production of the season, sourdough or yogurt is used as a starter culture due to the fact that fresh boza is not available. When sourdough is used, less viscous and a more acidic product is obtained compared to the product that is inoculated with prior boza product. When yogurt is preferred, a viscous and more acidic product is produced. In this product, the characteristic yogurt taste can be easily detected (Arici and Daglioglu, 2002; Holzapfel, 2015).

7.3.2 Composition and Nutritive Value of Boza

Boza is known as a source of carbohydrate, protein, fiber, and vitamins such as thiamine, riboflavin, pyridoxine, and niacin (Arici and Daglioglu, 2002; Kabak and Dobson, 2011; Baschali et al., 2017). Differences in the composition and nutritive value of boza are the result of the different types and amounts of cereals used and the spontaneous fermentation conditions. The selection of cereals to be used is very important because they affect the degree of fermentability, viscosity, and dry matter contents (Altay et al., 2013; Baschali et al., 2017).

It was reported that the chemical properties of boza were affected by types of raw material used during fermentation and storage time in the study of Akpinar-Bayizit et al. (2010). Total titratable acidity with regard to lactic acid was determined to be lowest in millet boza with 0.32% and highest in wheat boza with 0.61% because of the high fermentable carbohydrate content of wheat compared with other raw materials. The pH values changed between 3.43 and 3.86. During storage, the acidity of the samples increases (max value was 0.68% at 192 h) with a decrease in the pH. Moreover, the alcohol content was low in wheat boza (0.46%) and the fluctuations in the alcohol contents were observed depending upon microbial and enzymatic activities during storage period. The main organic acids of boza samples were found to be lactic, oxalic, pyruvic, and acetic acids. Citric, orotic, and malic acids were also determined in some samples. The highest organic acids in the samples were oxalic and lactic acids. While wheat boza had the highest amount of oxalic acid (425.50 mg/kg), lactic acid (416.86 mg/kg) was the highest in rice boza. The production of organic acids is based mainly on the existence of homo- and heterofermentative lactic acid bacteria in fermented cereals (Akin, 1997; Erbaş et al., 2006; Akpinar-Bayizit et al., 2010).

The physicochemical characteristics of two different boza samples were evaluated by Heperkan et al. (2014). The values of dry matter, total sugar, protein, ash, total acidity, pH, water activity in boza sample obtained by using wheat and maize as raw material were 22.74%–24.07%, 16.0%–22.62%, 0.46%–0.56%, 0.24%–0.44%, 0.25%–0.27%, 3.47%–3.95%, and 0.91%–0.94%, respectively. The dry matter was determined 14.39%, total sugar 6.63%, protein 0.65, ash 0.17%, total acidity 0.30%, pH 3.68, and water activity was 0.92 in the boza sample obtained by using millet as raw material.

Yeğin and Üren (2008) have determined the dry matter and protein contents of 10 industrially produced and bottled boza samples. The pH values of the boza samples ranged from 3.16% to 4.02%, while the total dry matter values were between 0.50% and 0.99%.

In another study, it was found that the pH, viscosity, and glucose contents of boza samples varied from 3.2 to 3.4, 380 to 500 cP and from 7.1 to 2.3 g/L, respectively (Gotcheva et al., 2000).

7.3.3 Health Benefits of Boza Consumption

Boza is considered as a nutritious and healthy beverage due to its fat, protein, carbohydrate, fiber, vitamin, amino acid, and lactic acid contents. The last compound is widely known as having positive effects on the digestion system and intestinal microflora (Arici and Daglioglu, 2002; Osimani et al., 2015). Furthermore, the decrease in pH to ~3.0–4.0 provides optimal conditions for the enzymatic degradation of phytates during the production of boza. It helps the product to obtain its typical flavor and affects protein absorption positively. Also, it avoids the growth of pathogenic and/or spoilage bacteria (Kabak and Dobson, 2011; Osimani et al., 2015). The last case is also supported by the presence of bacteriocins the antimicrobial compounds that is produced by most lactic acid bacteria. Bacteriocins increase the shelf life of the product and indirectly contain health benefits (Deegan et al., 2006; Todorov et al., 2007; Todorov, 2010). Kancabaş and Karakaya (2013) reported that boza can be a source of ACE-inhibitory peptides and so it contrasts hypertension.

Both arabinoxylan (found in wheat and rye) and β-glucan (found in barley and oats) are prebiotic compounds that may contribute to the reduction of LDL-cholesterol levels and therefore may also contribute to a decrease in the risk of cardiovascular diseases (Goldberg, 1994; Jaskari et al., 1998; Schmidl and Labuza, 2000; Crittenden et al., 2002; Karppinen, 2003; Holzapfel, 2015). Also, the low glycemic index of oats and barley is quite beneficial to diabetes (Angelov et al., 2006; Holzapfel, 2015). β-Glucan induces the growth of beneficial bacteria related to the colon of human (Jaskari et al., 1998; Mazza, 1998; Holzapfel, 2015).

The use of starter cultures is not common in the boza fermentation. The manufacturers utilize back-slopping method with indigenous strains (Heperkan et al., 2014). Boza includes most of lactic acid bacteria and yeasts. Lactic acid bacteria described in the boza contain *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus coprophilus*, *Lactobacillus coryniformis*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus sanfrancisco*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*, *Lactobacillus mesenteroides* subsp. *dextranicum*, *Leuconostoc raffinolactis*, *Pediococcus pentosaceus*, *Leuconostoc oenios* (reclassified to *Oenococcus oeni*), *Weissella confusa*, and *Weissella paramesenteriodes*. Most of the identified yeasts in boza are *Candida glabrata*, *Candida tropicalis*, *Geotrichum candidum*, *Geotrichum penicilatum*, *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, and *Saccharomyces uvarum* (Gotcheva et al., 2000; Arici and Daglioglu, 2002; Todorov and Dicks, 2006; Botes et al., 2007). According to the study by Gotcheva et al. (2000), lactic acid bacteria were always predominant in the microbial flora of boza and an average lactic acid bacteria:yeasts ratio was equal to 2.4. Lactic acid bacteria species were identified in different proportions, of which 82% belong to the genus *Lactobacillus* and 18% to *Leuconostoc* while the yeast species identified belong to the genus *Saccharomyces* (47%), *Candida* (33%), and *Geotrichum* (20%).

Boza is also considered as a rich source of probiotic bacteria (Altay et al., 2013). Todorov et al. (2007) have evaluated the probiotic properties of *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus,* and *Lactobacillus pentosus*. These microorganisms when ingested, act on the intestinal microflora of the host providing beneficial effects (Gotcheva et al., 2000), namely, they are very beneficial for human gastrointestinal system.

7.4 Kefir

Kefir is a traditional fermented milk beverage originated in the Caucasus and Anatolia regions, which is produced by the biochemical functions of microbial species found in kefir grains as starter cultures (Farnworth, 2003; Leite et al., 2012; Dertli and Hilmi, 2017). Kefir is a beverage known by different names such as kefyr, kephir, kefer, kiaphur, knapon, kepi, and kippi in various countries all over the world, which is widely consumed in Russia, the countries of the ex-Soviet Union, and Eastern Europe (Caballero et al., 2003; Fuquay et al., 2011; Pogačić et al., 2013; Watson et al., 2017). It can be manufactured from different types of milk (goat, cow, sheep, buffalo, camel) and kefir grains (Tamang and Kailasapathy, 2010; Baschali et al., 2017). Currently, new varieties are also made by using milk substitutes such as soya, coconut, and rice milk (Tamang and Kailasapathy, 2010; Panesar, 2011; Altay et al., 2013; Baschali et al., 2017). Kefir grains are small, irregularly shaped, and gelatinous yellowish structure (Guzel-Seydim et al., 2000; Gul et al., 2015). Kefir is a viscous and self-carbonated beverage and has a smooth, slightly foamy structure and whitish color (Hui et al., 2004; Yüksekdağ et al., 2004; Altay et al., 2013). It is a fermented milk product. The difference between kefir and other fermented milk products is that kefir grains can be recovered with a slight increase in grain biomass after fermentation (Guzel-Seydim et al., 2011; Satir and Guzel-Seydim, 2016).

The origin of kefir based on prior to the written records. In ancient times, the eastern nomadic shepherds noticed that the milk which they carried in their pouches during their travels sometimes turned into a foamy beverage. This beverage was called “kefir” by the nomads. It is believed that the word comes from “keyif” meaning “joy/pleasure” in Turkish (Kurmann et al., 1992; Watson and Preedy, 2010; Gaware et al., 2011; Watson et al., 2017). Another story about this issue is that the Prophet Muhammad gave the kefir grains to the people who live in the northern mountains, and kefir grains were transferred from generation to generation to this day (Lopitz-Otsoa et al., 2006; Watson et al., 2017).

7.4.1 Manufacturing Technology of Kefir

Kefir can be made from any kind of milk or milk substitutes (Irigoyen et al., 2005; Otles and Cagindi, 2003 fermente icecekler-basucu). In addition, used milk can be pasteurized, unpasteurized, whole fat, low fat, skim, and no fat (Otles and Cagindi, 2003; Yildiz, 2009; Altay et al., 2013).

Kefir grains used for the production of kefir are a combination of bacteria and yeasts in a matrix of proteins, lipids, and sugars. This symbiotic matrix creates grains that look like cauliflower. Kefir grains vary from 0.3 to 2.0 cm or more in diameters and are characterized by a folded or uneven surface. They have a characteristic smell (Tamime, 2006; Shah, 2017). The microflora of kefir is complex, not constant, and composed of (a) bacteria—mesophilic and thermophilic lactic acid bacteria, acetic acid bacteria, and possibly contaminants (*Pediococcus*, *Enterococcus*, *Micrococcus*, *Escherichia*, and *Bacillus*), (b) yeasts (*Saccharomyces* spp., *Candida* spp., *Kluyveromyces* spp., *Mycotorula* spp., *Torulopsis* spp., *Cryptococcus* spp., *Torulaspora* spp. and *Pichia* spp.), and (c) mold (e.g., *Geotrichum candidum*) (Tamime et al., 2011).

Kefir grains include 86.3% moisture, 1.2% ash, 4.5% protein, and 0.03% fat. The dry matter of the fresh kefir grains is 10–16 g/100 g, which contain about 30 g/100 g protein and 25–50 g/100 g carbohydrate (Libudzisz and Piatkiewicz, 1990; Shah, 2017).

Kefir is produced by fermentative activity of kefir grains. Microorganisms in the grains grow rapidly in milk. They produce lactic acid and other flavor compounds and causes physicochemical changes with fermentation (Guzel-Seydim et al., 2011; Satir and Guzel-Seydim, 2016).

There are mainly three methods for manufacturing kefir: (i) kefir produced by fermented milk with grains (traditional method), (ii) kefir produced by using commercial starter cultures inoculated directly into the milk (industrial method), and (iii) the commercial process by the Russian or European method (Otles and Cagindi, 2003; Farnworth, 2005; Sarkar, 2008; Fuquay et al., 2011; Leite et al., 2012, 2013; Frias et al., 2017).

In the traditional method, kefir grains are directly put in the pasteurized and cooled milk and incubated by stirring for about 24 h at 25°C. At the end of the fermentation, the grains are separated from the milk by filtering with a sieve. Kefir grains can be dried at 25°C and stored under refrigerator conditions for the next inoculation. Kefir is ready for consumption during 6–8 days after it is stored at 4°C (Altay et al., 2013). In all 2%–10% of kefir grains can be used in the traditional method, but kefir production with 5% kefir grains has indicated to be optimum for volatile acid and ethanol production (Sarkar, 2008; Frias et al., 2017).

In addition, the milk ratio is critical to obtain a product with stable quality due to the fact that it affects the pH, viscosity, CO_2 content, final lactose concentration, and the microbiological profile of the final product (Leite et al., 2012, 2013; Frias et al., 2017). Garrote et al. (1998) suggested that a ratio of 1% if a viscous and not very acidic product is demanded and informed that a ratio of 10% generates an acid beverage with low viscosity and a more effervescent taste.

In the industrial method, different methods can be used, but all of them have basically same principle. The milk is homogenized to 8% dry matter and heated at 90–95°C for 5–10 min. After cooling to 18–24°C, 2%–8% of kefir cultures are inoculated in it. The fermentation takes 18–24 h. The coagulum is separated by using pomp and distributed into bottles. Kefir is stored at 4°C following maturing at 12–14°C or 3–10°C for 24 h (Koroleva, 1988; Otles and Cagindi, 2003). The industrial process utilizes direct-to-vat inoculation or direct-to-vat set kefir starter culture. For the production of probiotic kefir, *Bifidobacterium* spp., *Lactobacillus* spp., and probiotic yeast (*Saccharomyces boulardii*) can be used as adjunct cultures mixed with the kefir grains or kefir direct-to-vat inoculation cultures (Tamime, 2006; Tamime et al., 2011). Industrial types of kefir may be mixed with sugar and fruit juices or flavors (Tamang and Kailasapathy, 2010; Baschali et al., 2017).

In the Russian or European method, the kefir produced by the traditional method is filtered after the fermentation stage and added to pasteurized milk, which is as a mother or bulk starter culture at the rate of 1%–3% without cooling. They are fermented at 20–25°C for 18 h and then cooled to 4°C and packaged. The obtained kefir is stored at 4–6°C (Frias et al., 2017).

A product that has an acceptable kefir flavor and longer shelf life (up to 28 days) is obtained with industrial production; while kefir produced with grains have a shelf life of only 3–12 days. On the other hand, the kefir produced with starter culture may not have the same therapeutic and probiotic properties due to the small number of different microbial species present in it (Fuquay et al., 2011; Pogačić et al., 2013; Watson et al., 2017).

Generally, kefir can be consumed with meals or as a probiotic drink. It is suggested for consumption due to probiotic mixture of bacteria and yeast (Simova et al., 2002; Gul et al., 2015). Kefir's probiotic property originates from kefir grains or cultures containing various species (Wszolek et al., 2001; Witthuhn et al., 2005; Gul et al., 2015). The microflora of kefir and kefir grains are different because of their origin and production methods (Temmerman et al., 2004; Gul et al., 2015). In kefir, lactic acid bacteria including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus kefir*, *Lactobacillus kefirgranum*, *Lactobacillus acidophilus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus thermophilus*, and *Lactococcus lactis* ssp. *cremoris* are characterized. Yeasts such as *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Candida kefir*, *Saccharomyces cerevisiae*, *Saccharomyces kefir*, *Saccharomyces unisporus*, *Zygosaccharomyces rouxii*, *Torulaspora delbrus*, *Torulaspora delbrueckii*, and *Debarymyces hansenii* (Shah, 2017) have been identified.

7.4.2 Composition and Nutritive Value of Kefir

Physicochemical properties and quality of kefir are influenced by the microbial quality of kefir grains, the ratio of grain and milk, incubation time and temperature, mixing conditions, and storage temperature (Yildiz, 2009; Altay et al., 2013). The chemical composition of kefir in accordance with literature is summarized in Table 7.1.

Table 7.1 Chemical Composition of Various Kefir Samples

Component	Quantity	References
Moisture		
Kefir produced with goat milk	83.74–87.91 g/100 g	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk using starter culture	91.81% (w/w)	Kök-Taş et al. (2013)
Kefir produced with cow milk	88.67 g/100 g	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk using kefir grain	92.02% (w/w)	Kök-Taş et al. (2013)
Lipid		
Kefir produced with goat milk	3.79–5.73 g/100 g	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk	3.37 g/100 g	Satir and Guzel-Seydim (2016)
Protein		
Kefir produced with goat milk	3.69–5.21 g/100 g	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk using starter culture	3.45 g/100 mL	Kök-Taş et al. (2013)
Kefir produced with cow milk	3.57 g/100 g	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk using kefir grain	3.47 g/100 mL	Kök-Taş et al. (2013)
Lactose		
Irish kefir produced using kefir grain (diluted milk)	1.8%	Rea et al. (1996)
Ash		
Kefir produced with goat milk	0.77–0.78 g/100 g	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk	0.61 g/100 g	Satir and Guzel-Seydim (2016)
Total acidity		
Kefir produced with goat milk	0.71%–0.85%	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk	0.69%	Satir and Guzel-Seydim (2016)

Continued

Table 7.1 Chemical Composition of Various Kefir Samples—cont’d

Component	Quantity	References
Lactic acid		
Kefir produced with cow milk using starter culture	0.81%	Kök-Taş et al. (2013)
Kefir produced with cow milk using kefir grain	7905.96 μg/g	Gul et al. (2015)
Kefir produced with cow milk using kefir grain	0.89%	Kök-Taş et al. (2013)
Irish kefir produced using kefir grain (diluted milk)	0.5%	Rea et al. (1996)
Kefir produced with buffalo milk using kefir grain	6826.81 μg/g	Gul et al. (2015)
Citric acid		
Kefir produced with cow milk using kefir grain	2453.00 μg/g	Gul et al. (2015)
Kefir produced with buffalo milk using kefir grain	811.00 μg/g	Gul et al. (2015)
Ethanol		
Kefir produced with cow milk using kefir grain	111.3 mg/L	Kök-Taş et al. (2013)
Kefir produced with cow milk using starter culture	97.4 mg/L	Kök-Taş et al. (2013)
Acetaldehyde		
Kefir produced with cow milk using kefir grain	3.8 mg/L	Kök-Taş et al. (2013)
Kefir produced with cow milk using starter culture	3.9 mg/L	Kök-Taş et al. (2013)
pH		
Kefir produced with cow milk using kefir grain	4.55	Gul et al. (2015)
Kefir produced with cow milk using starter culture	4.49	Kök-Taş et al. (2013)
Kefir produced with cow milk	4.59	Satir and Guzel-Seydim (2016)
Kefir produced with cow milk using kefir grain	4.47	Kök-Taş et al. (2013)
Kefir produced with buffalo milk using kefir grain	4.26	Gul et al. (2015)
Kefir produced with goat milk	4.54–4.55	Satir and Guzel-Seydim (2016)
Kefir produced with goat milk	4.42	Simsek et al. (2017)

In kefir samples produced from raw and skimmed UHT milk, L- and D-alanine, D-proline, D- and L-valine, L-threonine, glutamic and aspartic acids, cysteine, and methionine were detected and quantitatively determined by Menestrina et al. (2016). Natural proteins are formed by L-amino acids, but D-amino acids have also been found in different natural samples like fermented drinks (Brückner et al., 1993; Menestrina et al., 2016). Also, it is known that racemization can occur during manufacturing steps of processed food, such as fermented drinks and dairy products, as well as in biotechnological products (Brückner and Hausch, 1989; Lüpke and Brückner, 1998; Menestrina et al., 2016).

Angiotensin converting enzyme (ACE) activity resulting from the release of low molecular peptides was found in kefir samples produced by commercial and conventional methods from goat milk (Quirós et al., 2005; Tamime et al., 2011; Simsek et al., 2017). ACE-inhibitory activity of kefir sample produced by the conventional method from goat milk was expressed as an IC_{50} value of 190.71 µg protein/mL (Simsek et al., 2017).

Kefir also includes vitamins, macroelements such as K, Ca, Mg, P, and microelements such as Cu, Zn, Fe, Mn, Co, and Mo (Sarkar, 2007; Ozer and Akdemir-Evrendilek, 2014; Frias et al., 2017). The vitamin and mineral contents of kefir are illustrated in Table 7.2.

Kefir's vitamin and amino acid contents increase through fermentation by biological enrichment (Kabak and Dobson, 2011; Melo and Silva, 2014; Baschali et al., 2017). The synthesis of B group vitamins increases with increasing biological value of milk during

Table 7.2 Vitamin and Mineral Contents of Kefir (Satir and Guzel-Seydim, 2016)

Vitamins	
Vitamin A	
Kefir produced with goat milk	2.82–6.5 mg/100 g
Kefir samples produced with cow milk	3.56 mg/100 g
Vitamin E	
Kefir produced with goat milk	0.025–0.198 mg/100 g
Kefir samples produced with cow milk	0.034 mg/100 g
Vitamin B1	
Kefir produced with goat milk	0.041–0.047 mg/100 g
Kefir samples produced with cow milk	0.052 mg/100 g
Vitamin B2	
Kefir produced with goat milk	0.116–0.193 mg/100 g
Kefir samples produced with cow milk	0.151 mg/100 g
Vitamin B3	
Kefir produced with goat milk	0.239–0.244 mg/100 g
Kefir samples produced with cow milk	0.159 mg/100 g
Vitamin B6	
Kefir produced with goat milk	0.029–0.038 mg/100 g
Kefir samples produced with cow milk	0.024 mg/100 g

Continued

Table 7.2 Vitamin and Mineral Contents of Kefir (Satir and Guzel-Seydim, 2016)—cont'd

Vitamins	
Vitamin C	
Kefir produced with goat milk	1.88–2.29 mg/100 g
Kefir samples produced with cow milk	0.43 mg/100 g
Minerals	
Calcium	
Kefir produced with goat milk	120.5–140.8 mg/100 g
Kefir samples produced with cow milk	104.2 mg/100 g
Sodium	
Kefir produced with goat milk	27.2–35.1 mg/100 g
Kefir samples produced with cow milk	31.7 mg/100 g
Potassium	
Kefir produced with goat milk	153.3–202.1 mg/100 g
Kefir samples produced with cow milk	143.3 mg/100 g
Magnesium	
Kefir produced with goat milk	13.5–16.1 mg/100 g
Kefir samples produced with cow milk	10.5 mg/100 g
Iron	
Kefir produced with goat milk	0.14–0.52 mg/100 g
Kefir samples produced with cow milk	0.27 mg/100 g
Copper	
Kefir produced with goat milk	0.02–0.04 mg/100 g
Kefir samples produced with cow milk	0.02 mg/100 g
Zinc	
Kefir produced with goat milk	0.50–0.76 mg/100 g
Kefir samples produced with cow milk	0.46 mg/100 g
Manganese	
Kefir produced with goat milk	2.12–2.50 mg/100 g
Kefir samples produced with cow milk	1.51 mg/100 g
Selenium	
Kefir produced with goat milk	0.011–0.027 mg/100 g
Kefir samples produced with cow milk	0.011 mg/100 g

the fermenting actions of kefir bacteria and yeasts. It has been demonstrated by many researchers that during kefir fermentation pyridoxine, folic acid, vitamin B12, and biotin are produced by the microbiota (Kneifel and Mayer, 1991; Liutkevičius and Šarkinas, 2004; Baschali et al., 2017). However, the production of these compounds depends on the type of milk and the microbiota composition (Ahmed et al., 2013; Baschali et al., 2017). For the enrichment of the kefir with vitamin B12, *Propionibacterium freudenreichii* strains could be added in the kefir microbiota (Van Wyk et al., 2011; Baschali et al., 2017).

Satir and Guzel-Seydim (2015) determined that the total phenolic content of goat kefir samples varied from 516.03 to 1359.32 mg of gallic acid equivalent/L (Satir and Guzel-Seydim, 2015). In this study, phenolic acids such as gallic acid, catechin, and epicatechin, were also identified in kefir samples. The major phenolic acid in kefir sample was epicatechin while the minor phenolic acid was caffeic acid. The epicatechin and caffeic acid levels in kefir made from Hair goats were 10.73 mg/L and 0.35 mg/L, respectively. Kefir made from cow milk included 1.85 mg/L of epicatechin and 0.27 mg/L of caffeic acid. Also, it was thought that these compounds might be the contributors to the antioxidant capacity of kefir. The TEAC values of the kefir made from goat milk were determined between 6.38 and 10.68 mM, and the ORAC values were in the range of 15.33–17.33 mmol trolox equvialent/mL. The TEAC value of the kefir made from cow milk was found to be 5.46 mM, and the ORAC values was 13.88 mmol trolox equivalent/mL.

Carbon dioxide (1–2 g/L), alcohol (0.08%–2% g/100 g), and ~40 aromatic compounds such as diacetyl and acetaldehyde are also produced by the fermentation of milk by the lactic acid bacteria, and the resultant yeasts indicate characteristic organoleptic properties related with kefir (Beshkova et al., 2002; Irigoyen et al., 2005; Kabak and Dobson, 2011; Watson et al., 2017).

7.4.3 Health Benefits of Kefir Consumption

Historically, kefir is a fermented beverage that has been recommended in the treatment of conditions such as gastrointestinal problems, hypertension, allergy, and ischemic heart disease. Scientific health benefits reported for kefir include antipathogenic activity, antitumor and anticarcinogenic activities, lactose maldigestion, increased vitamin B synthesis, antiinflammatory/immunomodulatory effects, and hypocholesterolemic effects (Fuquay et al., 2011).

Kefir is explained to act against the pathogenic bacteria *Salmonella*, *Shigella*, *Helicobacter*, *Clostridium difficile*, *Escherichia coli*, *Staphylococcus*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Proteus vulgaris*, *Micrococcus luteus*, *Streptococcus pyogenes*, *Listeria* *monocytogenes*, and against *Candida albicans* (Lopitz-Otsoa et al., 2006; Prado et al., 2015; da Silva Fernandes et al., 2017). Some of the lactic acid bacteria in kefir such as *Lactobacillus acidophilus*, *Lactobacillus casei* subsp. *casei* or *paracasei*, and *Lactobacillus plantarum* and yeasts in kefir like *Saccharomyces cerevisiae*, known as potential probiotics, are able to adhere to the intestinal mucosa due to their resistance to low pH and bile salts, and bacterial pathogens can produce bacteriocins and inhibit some intestinal pathogens (Miao et al., 2014; da Silva Fernandes et al., 2017).

In an animal model study related to the antitumor and anticarcinogenic benefits of kefir consumption, it has been claimed that kefir increased apoptosis and reduced tumor size and prevented lipid peroxidation and increased glutathione in rats with colonic abnormal crypt formation. Similarly, kefir extracts have been shown to inhibit the growth of breast cancer cells in vitro (Fuquay et al., 2011).

There are numerous health benefits of kefir caused by its important probiotic effects especially on gut health. For instance, kefir improves the cardiovascular disease risk profile of young adults (between 18 and 24 years) by reducing C-reactive protein increase because of improved kefir digestibility resulting from lactose reduction by fermentation (O'Brien et al., 2015; Saadi et al., 2017). The treatment of probiotic also protected neurogenesis and cognitive function in antibiotic treated mice by mainly supporting progenitor cell survival in the brain (Möhle et al., 2016; Saadi et al., 2017). For therapeutic uses of probiotics, the minimum recommended daily intake ranges from 10^8 to 10^9 cfu/g (van Loveren et al., 2012; da Silva Fernandes et al., 2017). Bacterial contents of kefir differ from 6.4×10^4 to 8.5×10^8 cfu/g and yeasts from 1.5×10^5 to 3.7×10^8 cfu/g. After fermentation, kefir contains 10^6 cfu/g of acetic acid bacteria (Bamforth and Ward, 2014; Shah, 2017).

It is known that the existence of D-amino acids in kefir is important because previous research had proved that they are utilized to cover metabolic demand against the complete absence of the L-form. For example, the use of D-amino acids in infants was searched and it was determined that both the D-alanine and D-valine were used for protein synthesis (Menestrina et al., 2016).

Several studies recommended that kefir could induce the mucosal immunity, but the mechanism of this was not clarified (Thoreux and Schmucker, 2001; Urdaneta et al., 2007). Additionally, kefir is a useful cholesterol-lowering product (St-Onge et al., 2000; Urdaneta et al., 2007). It increases the propionic acid in the fecal flora while not altering the cholesterol synthesis. Related to the cholesterol metabolism, fermented products have been shown to inhibit the increase of liver triacylglycerol and cholesterol levels, but have no effect on plasma cholesterol levels (Oda and Hashiba, 1994; Urdaneta et al., 2007).

7.5 Ayran (Yogurt Drink)

Ayran is a popular Turkish yogurt-based drink that has a specific salty taste (Yildiz, 2009; Baruzzi et al., 2016). The annual production of ayran is ~1 million tons and it is commonly consumed in Turkey (Köksoy and Kilic, 2003; Yildiz, 2009; Altay et al., 2013; Yilmaz et al., 2015). Plain yogurt drinks are also consumed in Central Asia, the Balkans, and the Middle East, while yogurt drinks with fruits and sweeteners are mostly preferred in Europe and the United States (Colakoglu and Gursoy, 2011; Shah, 2017). It is known in different countries by different names, for example "Dough" in Iran, "Shenina" in Jordan, "Laban Ayran" in Syria and Lebanon, "Moru" in South India, "Ayrani" in Cyprus, and "Laban Arbil" in Iraq (Yildiz, 2009; Baruzzi et al., 2016). Ayran is a dairy nonalcoholic fermented beverage, which has low viscosity. It is easily digestible and generally consumed during the summer months (Kabak and Dobson, 2011; Altay et al., 2013; Baschali et al., 2017). People view ayran as a refreshing drink in the heat. The difference of ayran from other fermented milk beverages is that it is a salty yogurt drink and does not contain fruit flavor (Fig. 7.3; Kristbergsson and Ötles, 2016).

During storage, the quality of ayran can deteriorate due to acidity development and serum separation. For a good mouthfeel, the optimum consistency and no serum separation during storage are the desired quality criteria in ayran. The shelf life of ayran is between 10 and 15 days under refrigerator conditions (Köksoy and Kilic, 2003).

7.5.1 Manufacturing Technology of Ayran

Ayran is traditionally produced by the addition of water and salt to yogurt or by adding yogurt starter cultures to standardized milk for fermentation on an industrial scale (Yildiz, 2009; Altay et al., 2013; Yilmaz et al., 2015).

Ayran is traditionally prepared by mixing yogurt with water (30%–50%) and salt (0·5%–1%). It is produced daily and consumed fresh (Altay et al., 2013; Baschali et al., 2017). Homemade ayran, which is not required to be packed, can be sold in restaurants, buffets, and pastries (Altay et al., 2013). This ancient practice is still applied in some Turkish villages. Salt is used for protecting the product and hiding its sour taste (Yildiz, 2009; Kabak and Dobson, 2011; Baruzzi et al., 2016). But, the poor hygienic conditions affect the final quality of ayran resulting in an unpleasant sour and bitter taste. In addition, these conditions cause whey separation in a few days, even at low temperature (Köksoy and Kilic, 2003; Baruzzi et al., 2016).

Industrial production of ayran can be carried out by two methods. It can be produced either by the addition of water to yogurt or by the

Fig. 7.3 Ayran.

addition of water to milk first and then fermentation of diluted milk (Koçak et al., 2006; Yildiz, 2009; Shah, 2017). First, the fat ratio of raw milk is standardized (1.5% for full fat, 0.8% for half-fat, 0.15% for fat free). In the first method, milk is homogenized and then pasteurized (Yildiz, 2009; Shah, 2017). It is possible to obtain ayran with good microbiological quality via pasteurization (Sen and Küplülü, 2004; Yildiz, 2009; Shah, 2017). Next, yogurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) are inoculated to pasteurized milk and samples are incubated until a pH 4.2–4.4 is obtained. The starter cultures can produce bitter peptides (Altay et al., 2013; Baschali et al., 2017). The fermented samples are cooled to end the fermentation and development of acidity (Köksoy and Kilic, 2003; Yildiz, 2009; Shah, 2017). Appropriate amount of water is added to fermented sample, until the total solid content reaches 8%. After that salt (0.5%) is added. In the second method, standardized milk is diluted with water until 8% of total solid content is obtained. After that salt (0.5%) is added. Ayran is bottled in glass or polypropylene or polystyrene plastic containers (Sen and Küplülü, 2004; Shah, 2017).

Generally, the microbial composition of homemade ayran is similar to that of yogurt (Altay et al., 2013; Marsh et al., 2014; Baschali et al., 2017). The bacterial population in industrially manufactured ayran is higher than in homemade ayran (Altay et al., 2013; Baschali et al., 2017). Selection of starter culture is crucial to rheological and textural properties of ayran (Yildiz, 2009; Altay et al., 2013). When some strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* are used as starter cultures, a bitter flavor can develop as a result of the production of bitter peptides. Starter cultures can continue to produce lactic acid during storage, causing an unpleasant sharp and acid taste (Ray, 2001; Altay et al., 2013). In ayran production, there are various factors that could influence the contents of yogurt bacteria (Akalın and Gönç, 1999; Altay et al., 2013). It has been determined that the pH can change with the levels of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. Depending on the increase in acidity, the quantity of *Lactobacillus delbrueckii* ssp. *bulgaricus* increases; the quantity of *Streptococcus thermophilus* has decreased because of the inhibition effect of acidity on *Streptococcus thermophilus* (Tamuçay-Özünlü and Koçak, 2010; Altay et al., 2013).

7.5.2 Composition and Nutritive Value of Ayran

The composition of ayran depends on the type of the milk used, the fat content of milk, and the dilution rate used (Altay et al., 2013; Baschali et al., 2017). A 100 g of Turkish standard of ayran contain 90.5 g water, 9.5 g total solids, minimum 8 g solids-not-fat, 1.5 g fat, 1.6 g lactic acid, maximum 1 g salt (optional), and is free from pathogenic microorganisms (Akin and Rice, 1994; Sen and Küplülü, 2004; Tamime and Robinson, 2007). In another study, the composition and nutritive values (approximate per serving) of ayran have been reported as protein (7.0 g), fat (2.7 g), carbohydrate (9.6 g), iron (0.2 mg), phosphorus (194 mg), cholesterol (18 mg), zinc (1 mg), sodium (710 mg), vitamin A (190 IU), thiamin (0.06 mg), riboflavin (0.30 mg), niacin (0.16 mg), and vitamin C (2 mg). When one portion of ayran is consumed, the amount of energy received by the body is 90 kcal (Kristbergsson and Ötles, 2016). Apart from these, the acetaldehyde content of the ayran is affected by different manufacturing techniques, but these differences can not be detected in sensory analysis (Koçak et al., 2006; Shah, 2017).

The functional properties of ayran can be developed. For example, Kök-Taş and Güzel-Seydim (2010) have manufactured ayran by adding inulin as prebiotic and *Lactobacillus acidophilus* and *Bifidobacterium* spp. as probiotic strains. They presented that the product had better taste, appearance, and higher Lactobacillus bacteria count compared to the normal ayran (Altay et al., 2013). In the study by Khalil, pomegranate juice was added to obtain a functional ayran. The product contained carboxymethyl cellulose for controlling texture. As a result, the addition of pomegranate juice increased the antioxidant activity of ayran, while it had adverse effects on its rheological characteristics and serum separation (Shah, 2017).

7.5.3 Health Benefits of Ayran Consumption

Ayran is a highly valued drink with its nutritive and functional properties. One of the health benefits of ayran is that in the fermentation stage, lactose, which is natural sugar of milk, is broken down and used as food by the bacteria. This provides digestion of ayran easier than its milk (Kristbergsson and Ötles, 2016).

In vitro study using human colon cells, it was stated that ayran had an antioxidant potential which could prevent DNA damage (Grishina et al., 2010; Baschali et al., 2017). Grishina et al. (2010) reported that ayran can possess the functional and healthy properties such as antioxidant and protective activities on intestinal cells, related to its high amount of natural mesophilic and thermophilic lactic acid bacteria.

Other nutritional benefits of ayran are derived from minerals such as potassium, calcium, phosphorus, magnesium, and from vitamins such as riboflavin, B12, A, D, and K, which are abundant in the its structure (Kristbergsson and Ötles, 2016).

7.6 Gilaburu Juice

European cranberrybush (*Viburnum opulus* L.) is the fruit of a deciduous shrub, reaching 1.3–3.5 m height (Altan et al., 2005; Velioglu et al., 2006; Sagdic et al., 2014). It belongs to Caprifoliaceae family originated in Europe, Northern Africa, North Asia, and Western Russia (Velioglu et al., 2006; Sagdic et al., 2014). However, it is widely distributed in Turkey. It is called as "gilaburu" in the middle-Anatolia region, especially in the city of Kayseri, Turkey (Ulger et al., 2012). Shiny red gilaburu fruits, which are about 8 mm in diameter, ripe at the end of summer. A single cluster constitute 30–40 berries which have thin skin, single seed, and spherical shape (Velioglu et al., 2006; Sagdic et al., 2014). These fruits have a special astringent taste (Yilmaztekin and Sislioglu, 2015; Baschali et al., 2017). The yield of gilaburu juice increases together with maturation and then fruits possess the appearance of a weak, pendulous umbrella (Velioglu et al., 2006; Sagdic et al., 2014). In Russia, many Siberian nations, and Ukraine, the fruits are a component of drinks, sauces, and cakes and have been applied in folk medicine (Velioglu et al., 2006; Česoniene et al., 2012).

The gilaburu juice is a nonalcoholic, fermented traditional beverage obtained from gilaburu fruit, especially for people living in Central Anatolia (Ulger et al., 2012). Sometimes sugar can be added to the produced juice to mask the astringent taste (Yilmaztekin and Sislioglu, 2015; Baschali et al., 2017).

7.6.1 Manufacturing Technology of Gilaburu Juice

For the preparation of the beverage, after washing gilaburu fruits with water, they are allowed to stand in tap water at a dark place and room temperature approximately for 3–4 months to spontaneously ferment (Sönmez et al., 2007; Yilmaztekin and Sislioglu, 2015; Baschali et al., 2017). The fruit of *Viburnum opulus* matures during this process. The ripened fruits are pressed to obtain gilaburu juice and this juice is diluted with tap water (1:4) before consumption. After that, sugar is added to the juice (Soylak et al., 2002; Altan et al., 2005). This juice is not acceptable because of its astringent taste that leaves a tactile sensation (Velioglu et al., 2006; Sagdic et al., 2014). This taste is thought to be reduced with a long fermentation time by the local people consuming it and this beverage can be consumed more favorably than the fresh versions (Sagdic et al., 2014).

The industrial production of gilaburu juice is not very common in Turkey, but is usually produced at home by artisan (Altan et al., 2005).

In gilaburu juice, various lactic acid bacteria species have been identified, including primarily lactobacilli, such as *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus paraplantarum*, *Lactobacillus hordei*, *Lactobacillus coryniformis*, *Lactobacillus parabuchneri*, *Lactobacillus buchneri*, *Lactobacillus pantheris,* and *Lactobacillus harbinensis*, as well as *Leuconostoc* such as *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides* (Sagdic et al., 2014; Baschali et al., 2017).

7.6.2 Composition and Nutritive Value of Gilaburu Juice

There are only a few studies about composition and nutritive value of gilaburu juice in the literature. For example, the properties of gilaburu juice were determined by Altan et al. are as follows: acidity 15.67%, soluble solids 8.5 °Brix, pH 3.23, density 1.021 g/mL, total reducing sugars 5.84%, protein (for 59.7°Brix concentrated juice) 1.54% (Altan et al., 2005). According to the study by Sagdic et al., gilaburu juice's pH was in the range of 3.36–4.44 (Sagdic et al., 2014). It was found that gilaburu juice is rich in acetic acid (Yilmaztekin and Sislioglu, 2015; Baschali et al., 2017).

Sönmezdağ et al. (2017) characterized the aroma composition of fermented gilaburu juice and determined a total of 47 aroma compounds including acids, alcohols, ketones, volatile phenols, aldehydes, furans, lactones, an ester a terpene, and a pyranone in the sample. Among the compounds identified, acids were quantified at highest followed by alcohols. Within these, isovaleric acid (2778.11 μg/L) was the major aroma compound of gilaburu juice, followed by butanoic acid (1689.03 μg/L) and 4-methyl catechol (1042.93 μg/L).

The total phenolic and anthocyanin contents of gilaburu juice were evaluated by Česoniene et al. (2012). It was observed that the total phenolic content of gilaburu juice was in the range of 804.2–1168.8 mg gallic acid equivalent/100 g and the total anthocyanin content changed from 24.3 to 51.3 mg cyanidin-3-glucoside equivalent/100 g. The predominant organic acid of gilaburu juice was determined as L-malic acid by Çam and Hişil (2007). Apart from this, the presence of oxalic acid and L-ascorbic acid were also identified and quantified. While the mean concentrations of total phenolic compounds of fresh gilaburu juice and pasteurized gilaburu juice were 351.26 and 330.40 mg gallic acid equivalents/100 mL, average EC_{50} values as antioxidant activity were 25.06 μg/mg DPPH, and 30.87 μg/mg DPPH, respectively.

7.6.3 Health Benefits of Gilaburu Juice Consumption

In recent research, it has been found that gilaburu fruit (*Viburnum opulus* L.), which the main ingredient of gilaburu juice, is rich in antioxidants and has antimicrobial properties (Andreeva et al., 2004; Altun et al., 2008; Sagdic et al., 2014; Baschali et al., 2017). Furthermore, gilaburu has many health benefits. For instance, it is very effective in many diseases such as heart disease, coughs and colds, digestive troubles, asthma, hysteria, cramps of the limbs or other parts in females (especially during pregnancy) (Altan et al., 2005; Zayachkivska et al., 2006; Van et al., 2009; Česoniene et al., 2012).

It is thought that the squeezed gilaburu juice, such as gilaburu fruit, has a preventive effect on kidney and menstrual and stomach cramps (Soylak et al., 2002; Ulger et al., 2012). As reported from the treatment of mice after 1,2 dimethylhydrazine-induced colon cancer, the juice of gilaburu can be chemopreventive in early stages of colon cancer (Ulger et al., 2012; Baschali et al., 2017). The juice is deemed to have a probiotic potential because of the fact that it contains several lactic acid bacteria species (Sönmez et al., 2007; Sagdic et al., 2014; Baschali et al., 2017).

7.7 Conclusion

Fermentation is one of the oldest biopreservation methods known by almost all people in the world. By fermentation, the shelf life of food can be increased and new products with better appearance and taste can be developed. Fermented products contain more nutrients such as enzymes, amino acids, and vitamins compared to nonfermented products. Fermented foods that are rich in nutrients have many positive effects on health. This ensures that fermented products are often preferred by the consumers.

Traditional nonalcoholic fermented beverages currently enjoy success in many markets. It has been observed that these type functional beverages have received increasing interest. The healthy beverage industry, the scientific community, and consumers will benefit from the knowledge of traditional nonalcoholic fermented beverages presented in this review. The awareness about these beverages will be increased.

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8

FERMENTED VEGETABLE BEVERAGES

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8.1 Introduction

Fermentation is one of the oldest form of preservation, which consists of transformation of the simple raw materials into value-added products by utilizing the phenomenon of the growth of microorganisms or their activities on various substrates. Fermentation process will result in the formation of alcohols, antibacterial compounds, acidulants, and improves digestibility of carbohydrates and protein which helps in improving the nutritional strength and flavor. Besides this, fermentation also helps to eliminate or reduce natural toxicants, antinutrients, cooking time, and fuel requirements. Nutritional roles of fermented foods and beverages include indirect contributions through subjective enhancement of appetite and they enhance the pleasure of eating (Parvez et al., 2006). It is believed that, first cucumbers were fermented in the Middle East around 2000 BC. Kimchi, Korean-style fermented cabbage, was thought to have originated in the primitive pottery age from the natural fermentation of withered vegetables in seawater (Lee, 2001). The fermentation process may be alcoholic fermentation, struck fermentation, or malolactic fermentation; where yeast is most commonly used in alcoholic fermentation while a partial fermentation occurs in the second type and lactic acid bacteria is utilized in the third type. Fermentation processes can be because of primary metabolites and microorganisms used which may be due to the formation of alcohol, carbon dioxide (yeast), acetic acid (*Acetobacter*), lactic acid (lactic acid bacteria), propionic acid (*Propinibacterium freudenreichii*), and ammonia and fatty acids (Bacillus, molds). Early research on the *lactobacilli* present in fermenting vegetables was done by Fred (Priem et al., 1927; Preuss et al., 1928). Carl Pederson studied the sauerkraut fermentation from the 1930s to early 1970s. The

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fermentation of vegetables can result in the increase in shelf life by preserving the food with the lactic acid, acetic acid, and alcohol, helps to modulate the digestive process, makes the food more palatable and digestable, and changes the flavor and taste of the food. In this chapter on the fermentation of vegetable beverages, besides the development of products, emphasis is made on the nutrients formation and the evaluation of fermented products.

8.1.1 Global Scenario on Fermented Products

Fermented foods and beverages are known to human being 5000 years back. Egyptians in 3200 BC were skilled in the art of preparing fermented beverages and leavened bread. The global market value of food fermentation-based food industry was estimated to be around 250 million US dollars (Joshi and Pandey, 2004). The marketed fermented products show that cheese and alcoholic beverages are the major products (Knorr, 1995). But, the present scenario reflects that fermented-based foods have gained not only the importance but also the market potential. The global scenario on fermented vegetable products is presented in Table 8.1. It gives an overview of various types of products, the microorganisms involved, and its use with the country of origin.

Globally, fermented foods are inherent components of diets as they usually improve flavor, aroma, and appearance which increases the appeal. There is undoubtedly a reemergence of traditional and regional fermented foods with newer perspectives. Currently, fermented foods are market driven because of its health benefits and well-being of consumers as well. Fermented foods are paving the way for development of novel functional foods which dictates the functionality of the product.

However, in developing countries as well as India, fermented vegetable as such usage is very less. But the manufacturing and usage of pickles is an age-old traditional method, later it is been commercialized so that it can meet the needs of the consumers. The primary vegetable products produced in the United States and Europe are cucumber pickles (Pederson, 1979); fermented olives in the United States, Spain, Portugal, Peru, and Chile (Abriouel et al., 2011); sauerkraut in Europe, the United States, Canada, and Australia (Johanningsmeier et al., 2007); pickled fruit and vegetables in Indian subcontinent, South East Asia, East Asia, Africa, and Europe (Girado and Mazas, 2000; Ozcelik and Ulu, 2002; Ishikawa et al., 2003; Montano et al., 2004; Wen et al., 2006; Panda et al., 2007, 2009; Yucel and Uren, 2008; Sosinska and Obiedzinski, 2011); Kimchi in Korea (Kim et al., 2012); while shalgam, Tursu, and hardaliye are traditional products of Turkey (Kabak and Alan, 2011), vinegar used internationally (Hur and Lee, 1998; Su and

Table 8.1 Global Scenario of Fermented Vegetable and Fruits Products

Product and Country/Region	Substrate	Microorganism(s) Involved	Nature and Use	References
A. Fermented fruits and vegetable products				
Burong mustala-Philippines	Mustard	*Lactobacillus brevis, Pediococcus cerevisiae*	Salad, side dish	Lee (1994) and Rhee et al. (2011)
Dakguadong-Thailand	Mustard leaf	*Lactobacillus plantarum*	Salad, salt side dish	Lee (1994) and Rhee et al. (2011)
Dhamuoi-Vietnam	Cabbage, various vegetables	*Leuconostoc mesenteroides, Lactobacillus plantarum*	Salad, side dish	Lee (1994) and Rhee et al. (2011)
Fermented Fruit Beverages-International	Peach, melon, grape, watermelon, cashew apple, pomegranate, pineapple, banana orange, papaya, passionflower fruit	*Bifidobacterium, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus delbrueckii*	Liquid drink	Li et al. (2009), Chanprasartsuk et al. (2010), Fu et al. (2010), Wegkamp et al. (2011), Mestry et al. (2011), Kretschmer and Moll (2011), Pereira et al. (2011), and Mousavi et al. (2011)
Gundruk-Nepal	Mixed vegetables	*Lactobacillus plantarum, Pediococcus pentosaceus*	Dried vegetable product	Tamang et al. (1988)
Kimchi-Korea, East Asia	Korean cabbage, radish, various vegetables, red pepper, mustard leaf	*Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum*	Fermented in brine	Kim et al. (1999), Pyo et al. (2000), Sim and Han (2008), Han et al. (2009, 2011), Cho (2010), and Lee and Lee (2010)
Pickled fruit and vegetables-Indian subcontinent South East Asia East Asia Africa Europe	Cucumber, lemon, mango, ginger, red pepper, Japanese radish, onion, garlic, carrot, sweet potato, cabbage, mustard, red turnip, in broccoli, cauliflower, cabbage	*Lactobacillus plantarum*, lactic acid bacteria, *Leuconostoc* species, *Lactobacillus casei, Leuconostoc mesenteroides*	Pickle	Girado and Mazas (2000), Ishikawa et al. (2003), Ozcelik and Ulu (2002), Montano et al. (2004), Wen et al. (2006), Panda et al. (2007, 2009), Yucel and Uren (2008), and Sosinska and Obiedzinski (2011)

(Continued)

Table 8.1 Global Scenario of Fermented Vegetable and Fruits Products—cont'd

Product and Country/Region	Substrate	Microorganism(s) Involved	Nature and Use	References
Sauerkraut-International	Cabbage	*Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus casei*	Used in salads, consumed raw or cooked with meat or sausages	Kuensch et al. (1992), Yu et al. (2001), Kristek et al. (2004), Kusznierewicz et al. (2008), Champagne et al. (2009), and Wiander and Korhonen (2011)
Vinegar-International	Blueberries, ginger, kiwi, orange, orange, apple, mango, passion fruit, carrot, tomato, pomegranate, pumpkin	Yeast, *Acetobacter*	Cooking	Hur and Lee (1998), Yae et al. (2007), Su and Chien (2007), Zhang (2008), Sahara et al. (2010), and Marques et al. (2010)
B. Beverages—alcoholic and nonalcoholic				
Beer, whisky-International	Barley, rye, corn, wheat, rice, sorghum, finger millet	*Lactobacillus* species, yeasts, molds, and *Bacillus* species	Alcoholic drink	Mwesigye and Okurut (1995), Iwuoha and Eke (1996), Sankaran (1998), Steinkraus (1998), Cai (2010), and Harlow and Phillips (2011)
Coffee, Cocoa, Tea-International	Coffee berries, coffee beans, tea leaves, cocoa bean	Lactic acid bacteria, yeasts, and acetic acid bacteria	Nonalcoholic drink, powder	Kunz and Murdia (1994), Fuchs (1995), Avallone et al. (2001), Cooper (2006), Oda et al. (2006), Anon (2008), Camu et al. (2008), Dario and Eskes (2009), and Campos et al. (2011)
Wine-International	Fruits, rice	*Saccharomyces cerevisiae, Schizosaccharomyces acidodevoratus, Saccharomyces uvarum, Oenococcus oeni, Lactobacillus plantarum, Leuconostoc oenos*	Alcoholic drink	Joshi et al. (1990), Pena et al. (2005), Cascales et al. (2005), Bayon et al. (2005), Muratore et al. (2007), Fan et al. (2009), Woo et al. (2010), and Jeon and Lee (2011)

Source: Compiled from Literature.

Chien, 2007; Yae et al., 2007; Zhang, 2008; Marques et al., 2010; Sahara et al., 2010); Burong mustala in Philippines (Rhee et al., 2011); Dha muoi in Vietnam (Nguyen et al., 2013); Ekung in India (Tamang and Tamang, 2009); Fu-tsai in Taiwan (Chao et al., 2009, 2012); Gundruk in India, Nepal, and Bhutan (Karki et al., 1983; Tamang et al., 2005); Jiang-sun in Taiwan (Chen et al., 2010); Mesu in India, Nepal, and Bhutan (Tamang et al., 2008); Sinki in India, Nepal, and Bhutan (Tamang and Sarkar, 1993; Tamang et al., 2005); and Sunki in Japan (Endo et al., 2008; Watanabe et al., 2009). There may be more than 5000 varieties of common and uncommon fermented foods and alcoholic beverages being consumed in the world today by billions of people as staple components (Tamang, 2010). Fermented fruits and vegetables are part of the human diet, but they constitute a minor 5% of dietary intake (Manas et al., 2014).

8.2 Fermented Vegetable Products

Vegetables are used in the human diet because of its richness in vitamins, minerals, antioxidants, and dietary fibers. The variety of plant-derived foods that adds various phytochemicals with diversified health properties can be a part of diet. This strategy and practice maximize the prevention of age-related diseases, such as cardiovascular diseases, cancers, neurodegenerative diseases, and osteoporosis. In the modern years, consumption of more vegetables is strongly recommended for the natural supply of nutrients and dietary fiber for better intestinal health. In general, major part of the vegetables is consumed by humans as fresh, minimally processed, blanched, or cooked by boiling in water or microwaving. Fresh vegetables have a short shelf life and processed vegetables have limited shelf life. They undergo rapid microbial spoilage and the cooking brings about changes in chemical composition and physical characteristics. Fermentation of vegetables and fruits may not only improve safety and prolong shelf life, but also provide health benefits. Therefore, fermentation of vegetables is of great concern in this era.

The primary fermented vegetable products confine to pickles, fermented cabbage, that is, sauerkraut. Later, a series of vegetables, viz., mustard leaf, radish, red pepper, ginger, mango, onion, garlic, carrot, sweet potato, turnip, broccoli, cauliflower, pumpkin, tomato, ashgourd, bittergourd, etc., have been utilized for fermentation process, either to consume as vegetable or as juices. Lactic fermentation using *Lactobacillus* species is the most common process adopted, however, with the advancement in research, other microflora such as *Leuconostoc*, *Acetobacter*, *Pediococcus*, and *Bifidobacterium* species and yeast are utilized for fermentation of vegetables. Tamang et al. (2016) have reviewed the diversity of microorganisms in global fermented foods and beverages and presented the scenario of microorganisms isolated from vegetable products. However, the microorganism species and the period of fermentation process vary with the type of vegetable, form of vegetable, that is, vegetable as such or extracted juice as well as the temperature adopted for fermentation. Vegetables such as gourd, cabbage, and celery were fermented for 4, 7, and 9 days, respectively, using *Lactobacillus* species (Kuchta et al., 1994) while, Sapundzhieva et al. (1994) have reported 24 and 42 h for carrot and celery, respectively, when fermented at 37°C with 2% starter culture. Desai and Sheth (1997) studied the controlled fermentation of brined beetroot, carrot, cabbage, cucumber, cauliflower, ginger, green chilli, onion, radish, and turnip using six isolates of lactic acid bacteria. They found 4 days at 28–30°C was desirable for fermentation and could be preserved at 28–30°C by adding 0.1% sorbic acid to the brine. The natural fermentation of cucumber in brines showed a maximum lactic acid bacteria count with 5–8 days of fermentation with the decreasing trend later, while yeast growth was observed at the end of fermentation (Ic and Ozcelik, 1999). The fermentation of brined cucumber at 22–26°C for 39 days, cabbage and red beet at 28–45°C with the starters at 5×10^6 to 1×10^7 cells/g of vegetable, results in juice, with added sugar, salt followed by sterilization could store the juice for 1 year at 20°C (Afanaseva et al., 1992). Kraevska et al. (1996) developed lactic vegetable purees and beverages using *Lactobacillus plantarum* along with carrot, celery, and pumpkin puree. The fermented puree had greater levels of lactic acid (8 g/kg) and the beverages were found to be having pleasant smell, balanced sugar-acid ratio. The effect of salt content (2.5%), fermentation temperature (18°C, 14 days and 25°C, 7 days) and lactic starters was studied on sauerkraut fermentation (Yu et al., 2001), fermentation of white cabbage at 0.5% salt (Viander et al., 2003; Wiander and Ryhanen, 2005), 20 h at 0.5% salt (Wiander and Korhonen, 2011) have been reported. The effect of temperature (22–32°C) and salt concentration on lactic acid fermentation of radish showed an optimum temperature of 26°C and 2.5% salt for optimal sensory properties (Sharma and Joshi, 2007) and radish-based kimchies has been studied by Cho (2010). The lactic acid bacteria-based fermentation has been reported for cherry tomatoes at 40°C, 15 h (Chunling et al., 2009). Cucumber pickles at 20°C with 6 months shelf life (Ozcelik and Ulu, 2002); akakabu-tsuke-Japanese pickle, red turnip pickle (Ishikawa et al., 2003); longans, mustard pickle (Wen et al., 2006; Weng et al., 2011); and sweet potato pickles (Panda et al., 2007) have been reported.

Fermentation of vegetables not only improves/modifies the flavor of products, but also aids in the formation of minor nutrients. Hozova et al. (1993) have reported the increased level of folacin from 0.077 to 0.46 ppm, pantothenic acid increases by 30% in fermented cabbage. Uma et al. (1998) have found an enhanced level of fat, pyridoxine, and ascorbic acid in the fermented bittergourd and fenugreek with the better acceptability of the product. Vitamin B_{12} was formed in fenugreek as a result of the fermentation. In fermented garlic pickle, riboflavin, α-tocopherol, and amino acids increased while thiamine level decreased (Montano et al., 2004). Phenolic acids and antioxidants could be better preserved in fermented mustard pickle (Fang et al., 2008). Antioxidant activity in red pepper seed kimchi extracts (Sim and Han, 2008), cabbage (Kusznierewicz et al., 2008) increased on fermentation process. During ginger paste fermentation, the active component gingerol has decreased during fermentation process, and generated lemon-like flavor (Ku et al., 2010; Chun and Chung, 2011).

8.3 Fermented Vegetable Beverages

8.3.1 Scenario on Fermented Vegetable Beverages

Vegetable juices are getting more attention due to the presence of phytochemicals and nutritional value. In particular, fermented vegetable beverages have become popular for their health promoting attributes including the prevention of gastrointestinal ailments. The consumption of fermented vegetable juices has increased globally (Kopec, 2000). The convenience, pleasure, ethnic fusion, tradition, well-being, and mainly the health benefits are the encashing points of vegetable beverages. The concept of liquid form is either as vegetable juice or as a vegetable beverage. Beverages are made from the extraction of the liquid from fruit and vegetables and further optimized with minor ingredients for the fermentation process. The approach can be followed in two ways, one by fermentation of the vegetable followed by extraction of juice and the other by extraction of vegetable juice followed by fermentation.

Several reviews on various aspects of vegetable juices are available and an overview of those has been brought out in a comprehensive manner. The advantages of lactic acid bacteria fermentation and its importance of promoting human health have been reviewed (Soomro et al., 2002; Karovicova and Kohajdova, 2005; Chelule et al., 2010; Sook et al., 2011; Fred et al., 2013; Raffaella et al., 2013; Manas et al., 2014; Manas and Ramesh, 2015; Gitanjali and Mandal, 2016; Mduduzi et al., 2016). The functionality of foods with reference to health benefits has been described (Alan et al., 2014; Eva et al., 2014; Maria et al., 2014; Nguyen, 2015). However, Maria et al. (2017) while reviewing the health benefits of fermented foods, they are of the belief that there is a need for additional fundamental research on different fermentation associated with different microbial strains, their properties, and randomized controlled clinical trials to emphasize the repeatable effects of the fermented foods on human health.

The fermented vegetable products as well as the traditional beverages of varied countries have been reviewed. It covers the beverages of Turkey (Huseyin et al., 2008; Kabak and Alan, 2011), Korean fermented foods (Kim et al., 2012; Jayanta et al., 2016), Indonesian fermented foods by Ingrid (2016), African fermented foods (Eliud et al., 2016; Folarin et al., 2016; Mduduzi et al., 2016), and Indian fermented foods (Gitanjali and Mandal, 2016; Gitishree et al., 2016). The replacement of artificially produced beverages by the fermented foods has been highlighted by Jeroen (2013). The updated inventory on microorganisms used for fermentation covering a wide range of food matrices has been covered by Francois et al. (2012), where as functional properties of microorganisms in fermented foods have been described by Tamang et al. (2016). Swati et al. (2017), in their book chapter gave an overview of technological innovations in processing of fermented foods. Stephanie et al. (2015) have reviewed the impact of probiotic products and the microorganisms isolated from those foods on gastrointestinal well-being, other health benefits, but opined that these have not been widely translated to global inclusion in food guides. They have presented the case for inclusion and challenge health authorities to consider advocating these foods globally.

Chukwuma and Ngozi (2017) have dealt with all type of fermented products in their review but, main emphasis is on the biohazards, that is, at times microorganisms used for fermentation may become harmful under certain undesirable conditions. The aspects covered in reviews may give a picture on the status of fermented products, but, the actual research papers give the realities and paves the way for further research. Thus, the work on vegetables as well as juices fermentation has been presented. Moreover, reviews are not found on nutritional aspects and the product evaluation through clinical trials.

8.3.1.1 Development of Fermented Vegetable Beverages

Some of the researchers have followed the vegetable fermentation and used for juice or beverage processing. Generally, lactic acid fermentation is utilized and at times yeast fermentation also finds its place. The type of fermentation may be by using natural microflora or starter cultures. *L. plantarum* cultures can produce pectolytic enzymes such as polygalactranose, pectinylase, and pectin esterase (Sakellaris et al., 1988) which aids in production of better quality juice. At times, the low-acid vegetable juices such as carrot juice pose the problem of storage due to bacterial spoilage. Then, lactic acid fermentation aids in preservation of juice. In plant and vegetable fermentations, the use of lactic acid bacteria and their action helps in conversion of phenolic compounds such as flavonoids to biologically active metabolites via the formation of hydrolases, esterases, decarboxylase, and phenolic acid esterase (Filannino et al., 2015). The short-chain organic acids production during lactic acid fermentation also aids in development of fermented vegetable juices (Karovicova et al., 1992). The addition of varied species of *Lactobacillus*, *Leuconostoc* species, and *Propionobacterium* species to low salt-brined cucumber resulted in difference in juice formation (Chavasit et al., 1991), changes in organoleptic properties reflecting the necessity of optimization of process with reference of both the vegetable and the starter culture.

The fermented beverage from white cabbage and red beet with lactic cultures had a shelf life of 1 month at 4°C, while pasteurized juices had a good shelf life of 6 months at 4°C or 20°C (Nowakowskaja and Lipowski, 1996). Passos et al. (1997) have reported the use of lactic culture and fermentative yeast *Saccharomyces rosei* for fermentation of cucumber which helps in reducing excessive acid production by bacteria. Chinese cabbage leaves fermentation to prepare Kimchi juice was improved with the addition of sucrose or glucose, polysaccharide hydrolases (Chun et al., 1997). Larger beets were used for the production of probiotic juice with lactic cultures and higher quantity of lactic acid was produced by *L. plantarum* and *Lactobacillus acidophilus* than other cultures (Yoon et al., 2005). Kohajdova et al. (2006) in their study on the suitability of various vegetables for lactic fermentation reported that cabbages and courgettes are more suitable as compared to tomatoes and pumpkin. Kuboi (2006) research on fermentation of ginger juice inferred that fermented ginger juice can be used as a beverage ingredient. Smita et al. (2007) have tried the fermentation of anthocyanin-rich sweet potato by inoculating *L. plantarum* culture at 28°C into fresh juice and fermented for 48 h. Tamang and Tamang (2010) have attempted to produce khalpi and gundruk, ethnic fermented vegetable products using mixed lactic acid bacteria culture isolated from products. These products had higher sensory acceptance as compared to market products.

The effect of salt mixture of sodium, potassium, and calcium on fermentation performance of Spanish green table olives has been studied by Rodriguez et al. (2012) using central composite design. The product produced with salt mixtures was acceptable with the reduced sodium content in the final product. Optimization of fermented products although generally followed by permutation combination methods, the recent development is the use of design expert statistical software such as response surface methodology (RSM). RSM has been utilized for the optimization of ingredients by many researchers for substrate concentration, culture concentration, fermentation time, and fermentation temperature, for additives such as sugar and salt (Wadikar et al., 2010, 2011; Devaki and Premavalli, 2012a,b,d; Devaki, 2013; Rodriguez et al., 2012; Alina and Camelia, 2013; Alina et al., 2015). Shin and Kyoung (2014) showed the development of Tofu with Kimchi seasoning and lactic acid-based fermentation showed higher antioxidant activities, total phenolics, and free amino acids.

Tao et al. (2014) have reported the differences in growth and fermentative properties of four strains of lactic acid bacteria while developing Chinese sauerkraut. The salt concentration had a significant effect on sauerkraut fermentation at the early stage. The lactic acid bacteria population and metabolic rate decreased with an increased concentration (Tao et al., 2016).

8.3.1.2 Development of Fermented Vegetable Juices/Beverages

In general, the juice is extracted from the vegetables, then followed the fermentation process, mixing of juices, pasteurization, and addition of functional ingredient. Several researchers have attempted in this direction for the development of fermented beverages. Fedorova et al. (1993) developed the beverage from *Beta vulgaris* using lactic bacteria culture was acidic with an appealing aroma. Ozler and Kilic (1996) fermented turnip juice and its blends with lactic cultures and baker's yeast followed by pasteurization of juices, bottling, and storage. Although mixed juices had good sensory properties, red beet juice addition led to color deterioration during pasteurization itself, hence being not acceptable. Gorenkov et al. (1998) fermented the expressed juice with dried lactic acid bacteria (1%) for a period of 16–24 h and concluded that increased contents of amino acids, vitamin C, iron, and potassium with reduction in heavy metals were due to lactic fermentation. The mixed culture fermentation of fruit-vegetable juice with *Lactobacillus* species and *Saccharomyces cerevisiae* or *Leuconostoc mesenteriods* with *S. cerevisiae* at 30°C for 3 days showed that *Leuconostoc* mixed cultures were more suitable with reference to growth rate, acid production, and sensory properties (Kim et al., 1998; Cheigh et al., 1998). The role of propionic acid bacteria with lactic acid culture in the fermentation of red beet juice, sauerkraut in the formation of vitamin B_{12} and folacin has been reported by Babuchowski et al. (1999). However, Karovicova et al. (1999) have investigated the suitability of the *Lactobacillus* strain for fermentation of vegetable juices by selecting six strains for the study and it revealed that *Leuconostoc plantarum* and *Leuconostoc delbrueckii* were found to be acceptable. Cabbage juice fermentation with different starter cultures showed *L. plantarum* was best and 72–96 h of fermentation had the best flavor and aroma (Karovicova et al., 2002). The addition of garlic juice to cabbage has increased the sensory scores (Kohajdova and Karovicova, 2003) and 2% inulin prebiotic preparation addition to fermented cabbage juice markedly improved sensory properties of product (Kohajdova et al., 2003). Optimization of tomato juice fermentation with lactic cultures has been studied by Kohajdova et al. (2004). The fortification of cucumber juice with glucose, fructose, and the mixture during fermentation with *L. plantarum* has been studied by Lu et al. (2001) and found that relatively fructose fermentation continued resulting in an increase in lactic acid production. Buruleanu et al. (2012) have reported on the cucumber, white, and red cabbage juices with lactic fermentation. Sangeeta and Garg (2015) have shown spices addition in sauerkraut fermentation had better acceptability.

Functional vegetable beverage preparation was attempted by Filomena et al. (2008) using prebiotic components inulin and fructo-oligosaccharide to carrot juice fermentation by lactic cultures. A lactic acid beverage was prepared by fermenting tomato juice with *Lactobacillus* and *Streptococcus thermophilus* by fermenting at 42°C for 35 h and the final beverage was achieved with the optimal combination of 4% sugar, 4% honey, 0.06% citric acid, and 0.015% malic acid (Hua et al., 2009). Manea and Buruleanu (2010) had suggested that the fermentation of cabbage, carrots, and beetroot juices with lactic acid bacteria is preferable for obtaining optimum product as well as better sensory properties. Watermelon and carrot juice mixture fermented with *Lactobacillus acidophillus* has been reported by Mestry et al. (2011). Devaki and Premavalli (2012b) developed bittergourd fermented beverage (Fig. 8.1) using natural mixed culture of curd by

Fig. 8.1 Bittergourd fermented beverage.

statistical software by RSM for the optimization of the fermentation process with reference to curd concentration and the period. Through the software, the fermentation time of 30 h with 15% of curd was optimized. The authors reported that the quinine, which is the principle bitter component of bittergourd was reduced to 60% with fermentation process.

Pasquale et al. (2015) developed fermented cherry juice and broccoli puree and were found to be enriched with phenolic derivatives, high human bioavailability and biological activity, while Natalie et al. (2016) developed passion fruit juice. *Aegle marmelos* fruit juice fermented showed increased acidity, lactic acid, and DPPH (2,2-diphenyl-1-picrylhydrazyl) activity of 81% reflecting the antioxidant potential drink (Jayanta et al., 2017).

In the recent years, the concept of fermentation using lactic acid bacteria has been modified as probiotic drinks or nondairy probiotic drink for lactose intolerance consumers. Probiotic juices have been developed as a nondairy probiotic drink using lactic acid bacteria, and thus carrot juice (Shafiya et al., 2016), yellow carrot juice (Amr et al., 2016), blueberry and carrot juice (Carolina et al., 2016), cabbage and apple juice (Barbara et al., 2015), and oat-banana juice (Anna et al., 2016) have been developed. Nithya et al. (2017) have developed the probiotic cucumber juice with the optimized *L. plantarum* inoculum, prebiotic inulin, and stevia at 3%, 2%, and 3%, respectively, with 48 h of fermentation. The product had a pH 3.82, acidity 0.39%, sugars 128.3 lg/mL with a good shelf life at 4°C in polyethylene terephthalate bottles.

Besides using the pure cultures for fermentation process, attempts have been made for isolation of cultures from fermented fruits and vegetables. Lactic acid bacteria were isolated from kimchi juice (Kim et al., 1998) and radish (Kim and Choi, 2001). Kingston et al. (2010) have studied the lactic acid bacteria associated with beetroot and carrot fermentation and identified and characterized the genotypes using multiplex kPCR (polymerase chain reaction) techniques. Silvia and Medana (2013) reported functional properties of lactic acid bacteria isolated from Romanian fermented vegetables and these could be used for the fermentation of certain foods. *Lactobacillus pentosus* strains isolated from naturally fermented Alorena table olives were found to be promising probiotics and showed growth capacity, survival under different environmental conditions, ability to aggregate with pathogenic bacteria, etc. (Beatriz et al., 2016) reflecting the functional properties of bacteria strains. Kang et al. (2016) isolated three strains of lactic acid bacteria from kimchi which can serve as probiotics. These isolated strains are used for the fermentation of other juices (Choi et al., 2009; Anna et al., 2016; Onofrio et al., 2016).

8.3.1.3 Development of Alcoholic Beverages

Saccharification and fermentation process results in the formation of ethanol which is also in practice by using the tubers and vegetable juices. The starter cultures vary depending on the end-product desirability, either the alcohol, vinegar, wine, etc. Nakamura et al. (1996) studied the saccharification and fermentation from artichoke tubers using crude enzymes from *Aspergillus niger* and fermentation by *S. cerevisiae* which produced more than 20% alcohol after 5 days at 30°C. Hur and Lee (1998) produced vinegar by fermenting autoclaved pumpkin juice with an *Acetobacter* starter culture and ethanol for 14 days at 20°C followed by aging at 10°C for 14 days and the flavor characteristics were similar to European wine vinegar. *S. cerevisiae* IR_2 was used for the alcohol production from waste onions juice and the color was red due to anthocyanin content (Horiuchi et al., 2000). The fermentation temperature and inoculum size during alcoholic and acetic fermentation were optimized for tomato vinegar production (Zhaojun et al., 2002). The purple sweet potato, red wine, and beer were developed by Ramesh et al. (2012) and Sandeep et al. (2015), respectively. Wine and beer contained essential antioxidants and acceptable sensory qualities. A study of Jose-Maria et al. (2008) showed that the production of ethanol from waste onions using *S. cerevisiae* could be used for the development of fruit-vegetable vinegar beverage. Orange vinegar was obtained by alcoholic and acetic fermentation of fresh orange juice. Fresh juices of carrot, tomato, and hawthorn were added to vinegar which resulted in a good flavored nutritious vinegar beverage. Vivek and Dasgupta (2011) have compared the wines from grape juice and from beetroot and carrot juice fermentation. They reported that pH, acidity, and alcoholic content were lower in the vegetable wine and taste panelists rated vegetable wine to be superior to grape wine. Guava fruit wine prepared by Reddy and Reddy (2011) and mixed fruit wine by Alloysius et al. (2015) were acceptable by the panelists. Guava fruit wine was found to have 7.3% alcohol, 150 mg/L methanol, and 287 mg/L higher alcohols. It also contained 25 mg/L acetaldehyde, 28 mg/L total esters, total fusel oil 287 mg/L, and 30 g/L proponal.

Devaki and Premavalli (2012a,d) and Devaki (2013) developed ashgourd fermented beverage using wet yeast (Fig. 8.2), dry yeast, standard culture, and natural culture from grapes juice. Design expert statistical software was used for the optimization of starter culture and the beverage was optimized with 3% dry yeast, 1% wet yeast, 30% grape juice, 1% culture and 3 days fermentation time, and the alcohol content was 4.6%, 1.7%, 0.1%, and 1.6%, respectively.

Although black carrot alcoholic beverage (Kocher et al., 2016) and honey+coconut milk wine have been developed, the sensory ratings

Fig. 8.2 Ashgourd fermented beverage.

were low (Tochukwu and Oyinloye, 2017). Folarin et al. (2016) have reviewed the potential of fruits and leafy vegetables for the production of vinegar, beers, and wines. Thus, the progress on the vegetable source for the development of alcoholic beverages is ongoing.

8.3.2 Changes in Nutrition Profile on Fermentation

Naturally, vegetables contain large amounts of minor nutrients. So far, vegetable is serving as good source of vitamins, minerals, dietary fiber, and at times anticancer compounds. Fermentation of vegetable juices further improve in the formation of minor nutrients, as well as the changes in desirable flavors, improve in digestibility and internal absorption. Many of the new compounds with health-modulating potential can also be formed with fermentation process. Lactic acid is one of the major metabolite that is synthesized in amounts often reaching over 1% in lactic acid bacteria fermentations. Iraporda et al. (2015) proved that lactic acid (or lactate) proved to reduce pro-inflammatory cytokine secretion of Toll-like receptor (TLR)-activated, bone marrow-derived macrophages, and dendritic cells in a dose-dependent manner. Lactate has also been proved that it alters redox status by reducing the reactive oxygen species burden in intestinal enterocytes (Kahlert et al., 2016). Therefore, fermented foods, which contain fractions of lactic acid, and other organic acids, reach the small intestine those cell products might provide a core benefit of those foods. Some of the microbial-derived products made during fermentation are typically strain dependent. Water-soluble vitamins such as folate, riboflavin, and B_{12} are synthesized from various nonvitamin precursors by certain bacteria in plant and dairy foods (Russo et al., 2014; Chamlagain et al., 2015). Fermentation also helps in synthesizing amino acids and derivatives with neurotransmitter (e.g., g-aminobutyric acid) and immunomodulatory functions (Becerra et al., 2015). In addition, certain secreted proteins and exopolysaccharides produced during food fermentations might serve as antioxidants (Li et al., 2014; Hong et al., 2015), prevent adhesion of pathogens to the intestinal mucosa (Chen et al., 2014), or confer immune-stimulatory (Makino et al., 2016) or hypocholesterolemic activities (London et al., 2014; Martoni et al., 2015). Some polysaccharides also act as prebiotics and are fermented by the intestinal microbiota to short-chain fatty acids (Salazar et al., 2015).

The vegetable fermentation along with nutrients formation so far has been compiled (Table 8.2). It clearly brings out the fact that the research work on nutrients formation is very much limited.

Sauerkraut juice had high contents of protein, vitamin C, β carotene, thiamine, riboflavin, and minerals, while fermented red beet juice had acetyl choline, protein, thiamine, riboflavin, and vitamin C (Afanaseva et al., 1992). The lactic fermentation of beets, carrot, and cabbage juices has led to the increased contents of amino acids, vitamin C, iron, and potassium, and for reduced contents of heavy metals (Gorenkov et al., 1998).

Horiuchi et al. (2000) studied on the production of alcohol from onion juice had high potassium and low sodium contents, hence suggested for the use in prevention of high blood pressure. The fermentation of carrot juice by lactic cultures increased iron solubility in the juice and was more pronounced with *Lactobacillus pentoses* than *L. mesenteriods*. Enzyme pretreatment increased the iron solubility further by 10% (Bergqvist et al., 2005). Rakin et al. (2007) showed that fermented carrot juice with brewer's yeast autolysate had higher contents of some minerals such as calcium, phosphorus, iron, and β-carotene. Fermented beetroot juice had higher contents of betanin and vitamin C. Demir et al. (2004) have also reported that fermented carrot juice will be delicious with high nutritional value. The lactic acid fermentation of two red beetroot varieties, that is, Czerwona Kula and Chrobry resulted in increase in color components. However, fermented Chrobry variety beetroot juice had higher betanidin (Czyzowska et al., 2006). Fermentation of pasteurized carrot juice by *Bifidobacterium* and *Bifido bifidium* showed the decreased concentration of α and β-carotene levels by 15%–45% (Yang, 2007). On the other hand, raw carrot juice fermentation by *L. mesenteriods* had improved color and stability of juice (Jo et al., 2008). According to Son et al. (2008), the polyphenol content and antioxidant activity of the fermented carrot beverage increased with the increase in beet extract in the beverage.

Table 8.2 Fermented Vegetable Beverages

Vegetable	Microorganism(s) Involved	Nutrients	References
Cucumber	*Bifidobacterium bifidum, Lactobacillus casei, Lactobacillus plantarum, Lactococcus diacetylactis, Leuconostoc mesenteroides, Leuconostoc oenos, Pediococcus pentosaceus, Propionibacterium shermanii, Propionibacterium pentosaceus*	Not reported	Chavasit et al. (1991)
Green pea and onion	Lactic acid bacteria	Production of short chain organic acids	Karovicova et al. (1992)
Cabbage, red beet, carrot, celery, tomato, and hot pepper	Lactic acid bacteria	Increased, protein vitamin C, thiamine, riboflavin, β-carotene, minerals	Afanaseva et al. (1992)
Carrot, celery and pumpkin puree	*Lactobacillus plantarum*	Maintained vitamin C and β carotene	Kraevska et al. (1996)
White cabbages and red beets	*Lactobacillus plantarum, Leuconostoc mesenteroides, Lactobacillus plantarum*	Not reported	Nowakowskaja and Lipowski (1996)
Artichoke tubers	*Aspergillus niger, Saccharomyces cerevisiae*	Not reported	Nakamura et al. (1996)
Turnip, black carrot, redbeet	*Lactobacillus plantarum, Lactobacillus brevis,* Bakers' yeast	Not reported	Ozler and Kilic (1996)
Beetroot	*Lactobacillus plantarum, Lactobacillus cellobiosus, Lactobacillus xylosus, Lactobacillus fermentum, Lactobacillus casei, Lactobacillus buchnerii, Streptococcus lactis*	Not reported	Gavrilova et al. (1997)
Cucumber	*Saccharomyces rosei*	Not reported	Passos et al. (1997)
Carrot	*Bifidobacterium*	Improved nutritional value	Park et al. (1997)
Red beet and carrot	Lactic acid bacteria	Not reported	Rasic (1998)
Beets, carrots, cabbages	Dried lactic acid bacteria	Increased amino acids, vitamin C, iron, potassium, glutamic acid, cysteine, lysine, and β-carotene	Gorenkov et al. (1998)
Pumpkin	*Acetobacter*	Increased flavor components	Hur and Lee (1998)
Carrot, cabbage, peas, cucumber, celery and fermented radish	–	Not reported	Lee et al. (1998)

Orange, apple, carrot, capsicum, tomato	–	Enriched with vitamins A, B, C, E, and fiber	Stern (1998)
Fruit-vegetable	Lactic acid bacteria strains isolated from kimchi—natural	Not reported	Cheigh et al. (1998)
Fruit-vegetable	Lactic acid bacteria strains isolated from kimchi—natural	Levels of vitamin C and carotene stabilized, produced organic acids	Kim et al. (1999)
Red beet	*Propionibacteria* and lactic acid bacteria	Increased vitamin B_{12}, folacin	Babuchowski et al. (1999)
White cabbage and carrot	*Lactobacillus plantarum*, *Lactobacillus plantarum* *Lactobacillus delbrueckii*	Reduced nitrates	Karovicova et al. (1999)
Radish	Natural lactic acid fermentation	Not reported	Kim et al. (1999)
Onion	*Saccharomyces cerevisiae*	Increased potassium and lowered sodium contents	Horiuchi et al. (2000)
Carrot, apple, celery, watercress, jujube, and lycci	Lactic acid bacteria were isolated from traditionally made dongchimi—natural	Not reported	Kim and Choi (2001)
Cabbage, carrot, beet and onion	Lactic acid bacteria	Not reported	Gardner et al. (2001)
Cucumber	*Lactobacillus plantarum*	Increased lactic acid	Lu et al. (2001)
Red beet	*Paracoccus denitrificans*	Reduced nitrates	Tomczak and Czapski (2002)
Cabbage	*Lactobacillus plantarum,* mixed culture of *Lactobacillus plantarum,* and *Saccharomyces cerevisiae*	Decreased reducing sugars	Karovicova et al. (2002)
Cabbage and garlic	*Lactobacillus plantarum*	Increased organic acid	Kohajdova and Karovicova (2003)
Vegetables	Lactic acid bacteria, propionic acid bacteria	Folate increased, vitamin B_{12} formation	Agerstada et al. (2004)
Sugar beet	*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii*	Not reported	Klewicka et al. (2004)
Carrot	Lactic acid bacteria	Not reported	Demir et al. (2004)
Tomato	Lactic acid bacteria	Not reported	Kohajdova et al. (2004)
Beetroot and carrot	Brewer's yeast autolysate	Increased vitamin C, calcium, phosphorus, iron, β-carotene	Rakin et al. (2004)
Carrot	*Leuconostoc mesenteroides*, *Lactobacillus pentosus*	Increased iron	Bergqvist et al. (2005)
Beet	*Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus casei*, *Lactobacillus acidophilus*	Not reported	Yoon et al. (2005)

(Continued)

Table 8.2 Fermented Vegetable Beverages—cont'd

Vegetable	Microorganism(s) Involved	Nutrients	References
Cabbage, carrot, celery, and beetroot	*Lactobacillus plantarum*	Not reported	Karovicova and Kohajdova (2005)
Cabbage	*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii*	Not reported	Yoon et al. (2006)
Red beetroot	Lactic acid bacteria	Not reported	Czyzowska et al. (2006)
Carrot	Lactic acid bacteria	High in nutritional value	Demir et al. (2006)
Cabbage, tomato, pumpkin, and courgette	*Lactobacillus plantarum*	Not reported	Kohajdova et al. (2006)
Multivegetable	*Propionibacterium*	Not reported	Radyko et al. (2006)
Carrot, apple, and tomato	*Lactobacillus bulgaricus* and *Streptococcus thermophilus* derived from yogurt and *Lactobacillus plantarum* from pickles—natural	Not reported	Feng and Ying (2006)
Ginger	Yeast	Not reported	Kuboi (2006)
Carrot	Lactic acid bacteria	Vitamins A, C, B group, calcium, potassium, iron	Baysal et al. (2007)
Beetroot and carrot	*Lactobacillus acidophilus,* Brewer's yeast autolysate	Increased calcium, phosphorus, iron, β-carotene	Rakin et al. (2007)
Carrot	Commercial starters and isolated lactic acid bacteria strains	Not reported	Wiander and Ryhanen (2007)
Cucumber and onion	*Lactobacillus plantarum*	Not reported	Kohajdova et al. (2007)
Sweet potato	*Lactobacillus plantarum*	Not reported	Smita and Ramesh (2007)
Carrot	*Bifidobacterium lactis, Bifido bifidum* strains	Decreased glucose, sucrose, α and β carotenes	Yang (2007)
Celery and beetroot	*Bifidobacteria* culture	Not reported	Moraru et al. (2007)
Carrot	*Leuconostoc mesenteroides*	Not reported	Jo et al. (2008)
Onion	*Saccharomyces cerevisiae*	Not reported	Jose-Maria et al. (2008)
Carrot and beet	*Leuconostoc mesenteroides* isolated from carrot juice	Increased polyphenol content and antioxidative activity	Son et al. (2008)
Mixture of vegetables	Lactic acid bacteria	Not reported	Furuse et al. (2008)
Cabbage, radish, and celery	Lactic acid bacteria	Not reported	Buruleanu et al. (2008)
Carrot, tomato	Yeast and *Acetobacter*	Not reported	Zhang (2008)

Carrot	*Lactobacillus rhamnosus, Lactobacillus bulgaricus*	Not reported	Filomena et al. (2008)
Sugar beet	Lactic acid bacteria	Not reported	Visser et al. (2009)
Tomato	*Lactobacillus, Streptococcus thermophilus*	Not reported	Hua et al. (2009)
Carrot and amla	–	Not reported	Sahota et al. (2009)
Carrot and beetroot	Lactic acid bacteria	Not reported	Buruleanu et al. (2009)
Onion	Lactic acid bacteria isolated from various fruits and vegetables—natural	Not reported	Choi et al. (2009)
Tomato	–	Not reported	Jang et al. (2010)
Tomato	*Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis*	Increased lycopene content	Koh et al. (2010)
Cabbage, carrots, and beetroot	Lactic acid bacteria	Not reported	Manea and Buruleanu (2010)
Beetroot	*Lactobacillus brevis, Lactobacillus paracas*ei 0920	Not reported	Elzbieta and Agata (2011)
Watermelon and carrot	*Lactobacillus acidophilus*	Increased flavor and nutrient content	Mestry et al. (2011)
Cucumber, white, and red cabbage	*Lactobacillus acidophilus*	Not reported	Buruleanu et al. (2012)
Beetroot, carrot, and celery	Lactic acid bacteria	Not reported	Alina and Camelia (2013) and Alina et al. (2015)
Ashgourd	Dry yeast Wet yeast Natural culture-grape juice *Saccharomyces cerevisiae* RHONE 2226	Increased B vitamins, vitamin C, total phenols, antioxidant activity. Flavor components formation	Devaki and Premavalli (2012a) Devaki and Premavalli (2014a) Devaki and Premavalli (2012d) Devaki (2013)
Bittergourd	Natural mixed lactic acid culture	Increased B vitamins, vitamin C, total phenols, antioxidant activity.	Devaki and Premavalli (2011, 2012b)
	Lactobacillus sporogenes (Sporlac)	Flavor components, organic acids formation, reduced bitter component quinine	Devaki (2013)
Red radish	*Lactobacillus*	Five flavonoids, total phenols	Jing et al. (2014)
Cabbage and apple	*Lactobacillus plantarum, Lactobacillus rhamnosus* (two strains), *Lactobacillus brevis,* and *Lactobacillus pentosus*	Not reported	Barbara et al. (2015)
Carrot	Lactic acid bacteria	Not reported	Amr et al. (2016)
Cabbage	Lactic acid bacteria	Bioactive compounds formation	Kalpana et al. (2016)

(Continued)

Table 8.2 Fermented Vegetable Beverages—cont'd

Vegetable	Microorganism(s) Involved	Nutrients	References
Carrot, onion, and tomato	Lactic acid bacteria	Not reported	Onofrio et al. (2016)
Onion, tomato, and strawberry	Lactic acid bacteria	Increased antioxidant activity	Corona et al. (2016)
Carrot	*L. acidophillus*, *L. plantarium*, *L. casei*, *Bifidum longum*	Increased protein content, reduced Carbohydrates	Shafiya et al. (2016)
Carrot and blueberry	*Lactobacillus reuteri*	Increased antioxidant activity	Carolina et al. (2016)
Cucumber	*Lactobacillus plantarum*	Not reported	Nithya et al. (2017)

Source: Compiled from Literature.

The study of the 10 lactic acid bacteria cultures for the fermentation of vegetables showed an increase in folate concentration and one of the mixed cultures was superior by doubling the folate concentration in the native bioavailable form as 5-methyl-tetrahydrofolic acid. The folate concentration varied from 50% to 75% during fermentation process. The propiono bacteria species produced vitamin B_{12} (Agerstada et al., 2004).

The carrot and beetroot juice mixture fermented by brewer's yeast was found to be rich in minerals, calcium, phosphorus, iron, and carotene (Rakin et al., 2004). Shalgam juice, a lacto fermented carrot juice had a high nutritional value with A, C, and B group vitamins and calcium, potassium, iron, and minerals (Baysal et al., 2007).

The fermented vegetable powders chutney mix based on carrot, radish, and cucumber powders with anardana and amchoor powders developed by Joshi et al. (2011) were acceptable better with anardana powder combination. Carrot with anardana powder had the highest acceptability. Vivek and Dasgupta (2011) have reported that vegetable wine from beetroot carrot juice fermentation was reddish brown in color, acidity at 1%, sugar 3.1%, and alcohol content of 10.6% ± 0.8% with a good taste.

Misako et al. (2012) have investigated the efficacy of production of vitamin B_{12}, folate, and thiamine from 180 isolates of lactic acid bacteria from Japanese pickle. Amongst those, two strains of lactic acid bacteria extracellularly produced high levels of folate and a different strain produced vitamin B_{12}. Some isolates showed tolerance to high concentrations of salts, alcohol, and low initial pH. Lactic acid fermentation not only serve as a promising route to improve the nutritional qualities of processed foods, but also enhances the antioxidant activity of tropical fruit juices there by exerts health promoting properties.

Amongst the four starter cultures used for the fermentation of ashgourd beverage in the study by Devaki (2013), B vitamins formation was very greater in natural culture fermentation which was followed by wet yeast, dry yeast, and standard culture (Table 8.3). Alcohol formation was almost similar in both standard and commercial wet yeast culture while it was less in concentration in natural fermentation and it was very high in dry yeast fermentation. Although, natural grape juice fermentation had a high score of 7.9 for overall acceptability, the others also ranged from 7.6 to 7.8 which records as near to very good. In general, the acceptability of fermented beverage was almost same irrespective of the type of culture used.

The relative efficiency of these cultures for the formation of nutrients was better understood when the uniform colony levels of 8×10^{10} cfu/g were considered for calculation from the base data of Table 8.3. The results indicated the relative effect that natural fermentation of ashgourd juice had higher influence on formation of the

Table 8.3 Comparison of Ashgourd Fermented Beverage Developed by Different Yeast Sources

Parameters (Per 100 mL)	Commercial Culture (Dry Yeast)	Commercial Culture (Wet Yeast)	Natural Culture (Grape Juice)	Standard Culture
Yeast (g)	3	1	30	1
Fermentation (days)	3	3	3	3
Total phenols[a] (mg)	28.4 ± 0.14	14.0 ± 0.20	21.5 ± 0.02	28.3 ± 0.26
Antioxidants[b] (%)	12.6 ± 0.30	6.5 ± 0.08	17.8 ± 0.13	9.7 ± 0.21
Thiamine (B_1, μg)	244.6 ± 3.23	83.0 ± 1.53	498.7 ± 4.50	28.3 ± 0.53
Riboflavin (B_2, μg)	78.6 ± 0.36	5.2 ± 0.03	597.0 ± 4.50	6.0 ± 0.21
Niacin (B_3, μg)	50.9 ± 0.89	320.0 ± 2.08	646.5 ± 2.52	80.0 ± 4.73
Pyridoxine (B_6, μg)	140.0 ± 1.76	620.0 ± 4.04	463.5 ± 3.80	210.0 ± 5.51
Vitamin C (mg)	0.2 ± 0.01	1.4 ± 0.01	1.2 ± 0.01	2.5 ± 0.03
Acidity (%)	0.2 ± 0.01	0.4 ± 0.01	0.3 ± 0.02	0.13 ± 0.02
Total volatiles (%)	0.0020 ± 0.0002	0.0035 ± 0.0001	0.06 ± 0.0008	0.011 ± 0.0015
Alcohol (%)	4.6 ± 0.15	1.7 ± 0.03	0.1 ± 0.01	1.6 ± 0.20
OAA[c] (score)	7.8 ± 0.15	7.6 ± 0.05	7.9 ± 0.05	7.8 ± 0.11

[a] Total phenol expressed as mg/100-mL catechol equivalents.
[b] DPPH free radical scavenging activity for 1-mL sample extract.
[c] Over all acceptability (OAA) scored on nine point hedonic scale.
Data from Devaki, 2013. PhD thesis. Available from: http://hdl.handle.net/10603/76454.

vitamins, as well as functional parameters such as total phenols and antioxidant activity. Further, it was followed by dry yeast in the formation of total phenols, antioxidant activity, thiamine, and riboflavin, where as higher formation of niacin and pyridoxine was influenced by wet yeast fermentation. However, optimal culture concentration was different for each culture.

Further, for better operational management in the industries, at constant 1% culture concentration nutrients concentration was calculated. The results revealed that the formation of vitamins both B series, except riboflavin and C was relatively higher in wet yeast fermentation, followed by standard culture, while, riboflavin formation was found to be higher in dry yeast fermentation and the increased formation of phenols and antioxidants was highest in standard culture fermentation followed by wet yeast fermentation. Considering the cost of cultures, approximately wet yeast costs minimum of 7 paise per g while, 20 paise per g for dry yeast and grape juice and Rs. 4000 per g for standard culture. Considering all the factors, wet yeast proves to be a more viable solution for product strength and commercial application. The optimization of starter culture, yeast, and fermentation period mainly focusing on nutritional parameters for ashgourd fermented beverage has been reported for the first time by these authors. Ashgourd fermented beverages were pasteurized in polypropylene bottles and stored at 5°C, 15–34°C, and 37°C, had a shelf life of 6 months at ambient conditions based on the functional, nutritional and quality parameters and microbial analysis during storage. Functional parameters considered, that is, total phenol content decreased from the level of 16.0 mg/100 mL to 13.2 mg/100 mL, 11.8 mg/100 mL, and 11.2 mg/100 mL when stored at 5°C, RT (room temperature) and 37°C, respectively, where as there was slight increase in antioxidant activity from 4.4% to 5.6% when the beverage was stored at 5°C, while at RT and 37°C there was reduction to about 3.8% and 3.6%, respectively. The nutritional parameters, that is, thiamine, riboflavin, niacin, pyridoxine, and vitamin C reduced from the initial levels of 80.0 μg, 5.2 μg, 320 μg, 620 μg, and 1.4 mg to 59.6 μg, 3.8 μg, 235 μg, 504 μg, and 0.6 mg per 100 mL of the beverage when stored at 5°C; 18.0 μg, 1.7 μg, 151 μg, 414 μg, and 0.3 mg per 100 mL of the beverage when stored at RT and 11.2 μg, 1.0 μg, 122 μg, 326 μg, and 0.12 mg per 100 mL of the beverage when stored at 37°C, respectively and it was found that storing the beverage at 5°C showed better retention of vitamins. The quality parameters considered, that is, total volatiles, alcohol, acidity, and overall acceptability showed that the beverage showed decrease in total volatiles from 0.035% to 0.027%, 0.020%, and 0.018% and alcohol from 1.6% to 1.0%, 0.9%, and 0.7% when stored at 5°C, RT and 37°C, respectively. The acidity increased to 0.61%–0.72% at the end of 6 month of storage. The ashgourd fermented beverage was very stable up to

6 months and based on the sensory parameters, the beverage had a shelf life of 6 months. Further analysis of ashgourd fermented beverage volatiles by gas chromatography-mass spectrometry (GC/MS), it was observed that concentration of two flavor components increased and seven more new flavor components which were not present in the initial samples of fermented beverage were developed during the storage period. The components identified were 1,2,cyclopentanedione; 1,8-cineone, terpan/zieneol; 3, cyclohexane 1-methanol; 4H pyran, 4-one, 2-3 dihydroxy 6 methyl; ethyl hydrogen succinate; furyl hydroxymethyl ketone; and thymine. The stored beverage had a pleasant aroma as per the sensory analysis by the panelists.

The fermentation of bittergourd juice was carried out by Devaki (2013), keeping the concentration of starter culture constant, the colony count was 1×10^6 cfu in 1 g of standard culture and 1×10^6 cfu per 10 g of curd.

The bittergourd fermented beverage developed with natural culture curd fermentation had quinine 287.91 mg/100 mL, total phenol of 39.73 mg/100 mL, antioxidant activity 54.17%, B series vitamins such as thiamine was 0.33 mg/100 mL, riboflavin 0.27 mg/100 mL, niacin 0.67 mg/100 mL, pyridoxine 0.27 mg/100 mL, vitamin C 33.51 mg/100 mL, and overall acceptability score of 8 on 9-point hedonic scale. The bittergourd fermented beverage with standard culture had quinine 406 mg/100 mL, total phenol of 22.0 mg/100 mL, antioxidant activity 46%, B series vitamins such as thiamine was 0.21 mg/100 mL, riboflavin 0.19 mg/100 mL, niacin 0.72 mg/100 mL, pyridoxine 0.32 mg/100 mL, vitamin C 29 mg/100 mL, and overall acceptability score of 7.1 on 9-point hedonic scale. It is noteworthy to mention that thiamine, riboflavin, and vitamin C formation was very greater in natural culture fermentation where as in standard culture fermentation it helped in the formation of niacin and pyridoxine (Table 8.4). When the functional parameters were considered, it was observed that total phenols and antioxidant activity was higher in natural culture fermentation. In the developed bittergourd fermented beverage, bitter component quinine was higher in standard culture and the high bitterness was reflected in over all acceptability scores as seen in Table 8.4, also natural culture fermentation had less bitterness and was highly acceptable with the score of 8 on 9-point hedonic scale. With these results, it was noticed that natural culture fermentation helped in formation of thiamine, riboflavin, vitamin C, and also quinine content was relatively lesser when compared to the standard culture, but the acceptability was rated very good with the score of 8.0 for natural culture fermentation, while for standard culture fermented product, the score was 7.1 with the rating as good. In order to understand the better relative efficiency of both cultures for formation of nutrients, nutrients have been calculated from the base data (Table 8.4)

Table 8.4 Comparison of Bittergourd Fermented Beverage Developed by Different Lactic Acid Bacteria Sources

Parameters (Per 100 mL)	Natural Culture (Curd)	Standard Culture (*Lactobacillus sporogenes)*
Quinine (mg)	287.91 ± 1.62	406.00 ± 3.5
Antioxidants[a] (%)	54.17 ± 1.15	46.00 ± 0.75
Total phenols[b] (mg)	39.73 ± 1.18	22.00 ± 0.00
Thiamine (B_1, mg)	0.33 ± 0.002	0.21 ± 0.03
Riboflavin (B_2, mg)	0.27 ± 0.002	0.19 ± 0.01
Niacin (B_3, mg)	0.67 ± 0.003	0.72 ± 0.01
Pyridoxine (B_6, mg)	0.27 ± 0.002	0.32 ± 0.01
Vitamin C (mg)	33.51 ± 0.62	29.00 ± 0.21
OAA[c] (score)	8.00 ± 0.10	7.10 ± 0.10

[a] DPPH free radical scavenging activity for 1-mL sample extract.
[b] Total phenol expressed as mg/100-mL gallic acid equivalents.
[c] Over all acceptability (OAA) scored on nine point hedonic scale.
Data from Devaki, 2013. PhD thesis. Available from: http://hdl.handle.net/10603/76454.

on uniform colony levels of 1×10^6 cfu/g. The results indicated that the standard culture fermentation had higher influence in formation of the vitamins, as well as functional parameters such as total phenols and antioxidant activity, while the bitter component quinine was very high when compared to the natural starter culture fermentation, curd. According to the overall acceptability score, natural culture fermentation scored high in comparison with standard culture. Further, for better operational management in the industries at constant culture of 1%, the calculated parameters showed that standard culture influenced more on nutrients formation and functional parameters, with the bitter component higher reflecting on overall acceptability score. Considering the cost of cultures, approximately it works out to be minimum for curd with 3 paise per g, while Rs. 4 per g of standard culture. Considering cost and acceptance, natural culture fermentation, that is, curd looks to be more viable solution for better acceptance and commercial application.

Bittergourd fermented beverage packed in self-standing polypropylene bottles had shelf life of 60 days at 5°C based on the functional, nutritional, and quality parameters and microbial analysis during storage. The functional parameters considered, that is, quinine, total phenols, and antioxidant activity showed decrease from the initial levels. Quinine content showed marginal decrease from the initial level of 307–303 mg, 301 mg, and 300 mg per 100 mL of the beverage when stored at 5°C, RT and 37°C, respectively. Total phenol decreased from 27.0 mg/100 mL to 15.0 mg/100 mL, 9.0 mg/100 mL, and 6.0 mg/100 mL and antioxidant activity decreased from 60.0% to 23.2%, 10.0%, and 7.0% when the beverage was stored at 5°C, RT and 37°C, respectively. The quality parameters showed increase in acidity and browning from 0.47% to 0.82% and 0.010–0.376, respectively, at the end of 60 days of storage at 37°C. The product was acceptable and stable up to 60 days when it was stored at lower temperature (5°C). The nutritional parameters considered, that is, thiamine, riboflavin, niacin, pyridoxine, and vitamin C showed reduction in all the vitamins. Thiamine showed decrease from 0.34 to 0.30, 0.22, and 0.19 mg; riboflavin decreased from 0.26 to 0.23, 0.09, and 0.07 mg; niacin from 0.70 to 0.64, 0.60, and 0.57 mg; pyridoxine from 0.21 to 0.18, 0.13, and 0.11 mg; and vitamin C from 30 to 25, 18, and 18 mg per 100 mL of the beverage when stored at 5°C, RT and 37°C, respectively. The analysis of flavor components of bittergourd fermented beverage by GC/MS has revealed that the initial sample had six flavoring components and was identified as phenyl ethyl alcohol, linalool, β fencyl alcohol, myrtenol, acetic acid, and 9-octadecenoic acid. During the storage period, four components were identified, that is, β terpene; dl-limonene; terpene-4-ol; and β fencyl alcohol. The initial flavor components except β fencyl alcohol were volatilized during storage and β terpene; dl-limonene; and terpene-4-ol were formed as new components after storage period. The bittergourd fermented beverage was analyzed for organic acids by high-performance liquid chromatography (HPLC) revealed the presence of six acids and the initial concentrations of ascorbic acid, fumaric acid, glucoronic acid, lactic, and quinic acid were 35, 12, 20, 10.4, and 179 mg, respectively, per 100 mL of the beverage. During storage, up to 60 days, the concentration of acids decreased by about 60%–70%, but the decrease was temperature-dependent reflecting higher losses at 37°C as compared to 5°C. However, the loss of lactic acid was revealed less. The product was stable up to 60 days when it was stored at lower temperature of 5°C, but it was not acceptable when stored at RT and 37°C because of the browning problem.

Pasquale et al. (2013) have found that *Lactobacillus* fermentation of pomegranate juice led the increased concentration of total polyphenolic compounds and antioxidant activity. Adetuyi and Ibrahim (2014) have investigated the formation of bioactive compounds during fermentation of okra seed and reported that during fermentation significant increase in phenolic content, vitamin C, and total flavonoids showed greater antioxidant activity. The evaluation of red radish brines fermented with lactic acid bacteria showed five flavonoids and the total phenolic content was 206–220 μg/mL (Jing et al., 2014). The properties of mustard leaf kimchi have been studied by Hye et al. (2016) and it revealed that minerals were highest up to 2 months and lactic acid up to 3 months of fermentation while vitamins decreased with fermentation. Wieslaw et al. (2015) have shown that red cabbage fermented is a good source of anthocyanins and antioxidant activity.

The development of nondairy fermented beverages with vegetable juices and kefir microorganisms resulted in an increase in number of volatile organic acids and alcohols while aldehydes decreased. In particular, the esters were present in high amounts in strawberry, onion, and melon juices whereas carrot and fennel registered an increase in terpenes and changes in color attributes was observed based on the vegetable. Strawberry, onion, and tomato juices retained high antioxidant activity after fermentation. The overall quality assessment revealed that carrot kefir-like beverage was relatively had higher acceptance by the panelists (Corona et al., 2016). Carolina et al. (2016) have shown the increased antioxidant activity during fermentation of blueberry, carrot juice blend by lactic acid bacteria fermentation for 40 h. Kalpana et al. (2016) have reported that fermentation of cabbage has led to the formation of potential bioactive compounds which are beneficial to health. The Spanish green table olives fermentation and packed samples analyzed for volatiles by GC/MS (Antonio et al., 2018) revealed that a total of 132 volatile compounds identified, the compounds were 10 phenols, 25 alcohols, 11 acids, 39 esters, 8 hydrocarbons, 14 carbonyl compounds, 17 terpenes, and 6 unidentified compounds. Although the nutritional aspects during fermentation have received less attention earlier, slowly it is getting the importance over the years.

8.3.3 Evaluation of Fermented Vegetable Beverages

The evaluation of the products refers to broadly acceptance of the product by the consumers, safety of the product, effectivity on the organs, or diseased conditions of the animal or human models. Otherwise called the health benefits of the products in human system. The fermented beverages besides providing better nutritional properties due to the transformation of substrates and formation of bioactive or bioavailable end products, improvement of sensory properties, shelf life, and safety, these beverages provide many health benefits beyond the starting food materials. The safety aspect is not an issue when the natural process of fermentation is carried out, however, the processing steps, culture purity, etc., should be taken care of.

8.3.3.1 Sensory Evaluation

The acceptance of the fermented vegetable beverage can be followed by sensory evaluation methods specifically the product evaluation on 9-point hedonic scale with the score of 1 as not liked and 9 as excellent. The product is generally evaluated for taste, aroma, flavor, color, and over all acceptance. Park et al. (1997) reported that fermented carrot juice by *Bifidobacterium* had higher sensory scores as compared to nonfermented carrot juice. The addition of garlic juice (0.1%) with lactic fermented cabbage juice had best sensory scores of acceptability (Kohajdova and Karovicova, 2003). The effect of inulin-based prebiotic preparation on fermented cabbage juice shows improved sensory properties. Demir et al. (2004) have shown that lactic fermented carrot juice with enzyme liquefaction had better sensory scores even after long-term storage of 6 months. Fermented cucumber juice with *L. plantarum* as starter culture and added onion juice showed highest scores of harmonized taste, aroma, and flavor (Kohajdova et al., 2007). On fermentation, reduction of pungency of onions (Roberts and Kidd, 2005), deodorization of garlic (Kakimoto et al., 1998), and reduction of pungency of pepper (Suh and Chang, 2002) have been reported.

Fermentation of carrots, French beans (Raffaella et al., 2008), and red and yellow peppers (Raffaella et al., 2009a) with the use of autochthonous mixed starter showed firmness and desirable fragrance in carrots and French beans while higher firmness and color index in peppers in unstarter samples. The different behavior of 10 autochthonous strains on tomato juice fermentation was observed with reference to glutathione, total antioxidant activity, and ascorbic acid and the principal component analysis showed large number of volatiles belonging to various classes differentiated the characteristics (Raffaella et al., 2009b). Sethuraman et al. (2010) evaluated the lactic acid fermented sweet potato pickle which had higher consumer acceptance. Vivek and Dasgupta (2011) have reported that the taste panelists have rated vegetable wine from beetroot and carrot juice as higher in taste, color, and appearance as compared to wine from grape juice.

Devaki (2013) reported that ashgourd and bittergourd fermented beverages were very well accepted after fermentation process by semitrained panelists with an acceptability score of 7.6 and 8 on 9-point hedonic scale. The sensory quality of the bittergourd fermented beverage was scored above acceptable range when compared with fresh bittergourd juice, showing that the bitterness reduced with the fermentation.

8.3.3.2 Animal Studies

The rat study on supplementation of fermented beetroot juice had showed the increased antioxidant status and its regular intake may reduce the risk of colon cancers induced by an inappropriate diet (Elzbieta et al., 2012). As reported by Devaki (2013), ashgourd fermented beverage was found to be highly acceptable by semitrained panel members and had shelf life of 6 months. The safety of the product was evaluated through animal experiments before supplementing it to human subjects. Fermented beverage was fed to male Wistar rats weighing 150–200 g for a period of 15 days and evaluated for physical appearance, liver enzyme test, and kidney functioning test. A total of 200 mL of fermented beverage was provided in bottles and were kept in the cages of rats. After the supplementation, physical appearance, liver enzymes, and kidney functioning test were carried out in both control and experimental group. Serum glumatic-oxaloacetic transaminase (SGOT) (u/L) was 24.32 u/L in control group and 23.69 u/L in experimental group (Fig. 8.3). Serum glutamic-pyruvate transaminase (SGPT) (u/L) was 16.92 u/L in control group and 20.48 u/L in experimental group (Fig. 8.3). Creatinine values were 0.3± 0.16 mg/dL in control group and 0.35± 0.18 mg/dL in experimental group (Fig. 8.4). Safety test revealed that the physical appearance was normal and SGOT and SGPT enzymes of liver and creatinine for kidney functioning were within the limits of normal range and proved that the product was safe and fit for consumption.

Jin et al. (2014) have conducted a clinical study on pigs with the fermented feed supplemented with carrot by-product, which showed increased daily weight gain, higher meat quality, quality grade, and water holding capacity as compared with basal diet, but, with the reduced *longissimus dorsi* muscles. Blanca et al. (2015) showed that

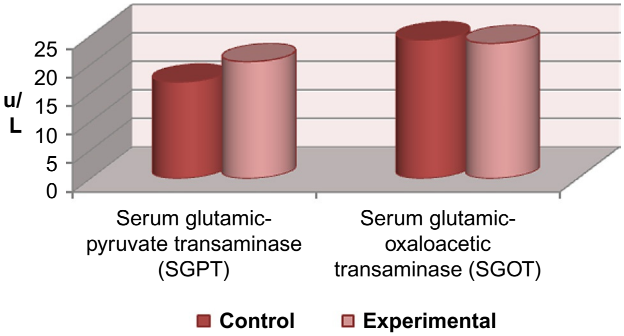

Fig. 8.3 Effect of feeding ashgourd fermented beverage on levels of liver enzymes on male Wistar rats.

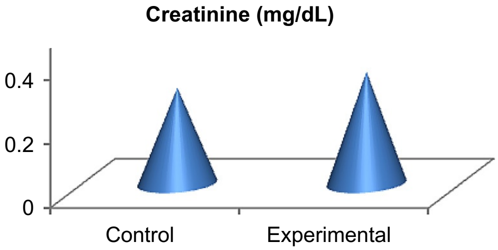

Fig. 8.4 Effect of feeding ashgourd fermented beverage on levels of creatinine on male Wistar rats.

administration of orange beverage to mice increased the levels of glutathione, uric acid, improved lipid metabolism, and decreased oxidized low-density lipoprotein (LDL) showing the probable protection against cardiovascular risk factors as compared to the control group. Kohei et al. (2015) in their rat-based clinical study showed that carrot, cucumber, eggplant, and pumpkin pickles administered orally reduced the blood pressure of spontaneously hypertensive rats. The preliminary study on the use of in vitro and in vivo features for safety and functionality evaluation of *Lactobacillus* strains isolated from kimchi has shown an encouraging response on decreasing body weight of obese mouse model over 8 weeks (Soyoung et al., 2016). Ji Won Choi et al. (2017) study revealed that fermented ginger extract administration into rats resulted in biotransformation of 6-shagol to 6-paradol and proved as a potential neuroprotective agent.

8.3.3.3 Human Clinical Trials

Product evaluation generally refers to the acceptability of the product by the consumers. Second, scientific evaluation confines to nutrients, microbial safety analysis. Third, biochemical evaluation normally includes the physiological role thereby the product impact on the physiological system in terms of human models is required to investigate, which otherwise refers to clinical trials of the product. Nuraida (2015) has provided general review on the health promoting lactic acid bacteria in Indonesian fermented foods and opined that human studies are still limited. Beer drinking effect on ultrasound bone mass in women (Juan et al., 2009) on pregnancy, breast feeding, menopause (Tirso et al., 2015), and reduced cardiovascular diseases (Armin et al., 2008) have been reported. Kim et al. (2011) have reported that consumption of kimchi for 2 weeks by obese and overweight individuals led to a decrease in body mass index (BMI), body fat, waist hip ratio, blood pressure, fasting blood sugar (FBS), and total cholesterol. However, Hong and Hae (2014) in their investigation on the association between hypertension and kimchi consumption with 20,114 participants found that even high consumption of kimchi did not show any association with an increased prevalence of hypertension in humans and probably high potassium intake from salted kimchi might have neutralized the effect of elevated sodium intake on blood pressure levels.

A study was conducted by Devaki and Premavalli (2012c) on geriatrics population to see the effect of ashgourd fermented beverage on anthropometric, nutritional, and biochemical status of institutional elderly inmates. Ashgourd fermented beverage (180 mL) was supplemented for 60 days to inmates of experimental group with the instructions to drink in fasting condition as a first drink in the morning and control group received no supplementation. The impact of supplementation on anthropometric data showed decrease in weight about

1–3 kg in the experimental inmates after the supplementation with the significance level of $P<0.05$, waist circumference showed reduction of 3–5 cm experimental inmates. Clinical manifestation and morbidity profile showed that glossitis, pallor, angular stomatitis, pale, and flat nails in experimental group were reduced by 4%, 26%, 3%, and 9%, respectively, and it showed 27% decrease in ganeto urinary disorder and gastro intestinal disorder by 4%. Biochemical profile showed that in experimental group, FBS, and postprandial blood sugar (PPBS) were reduced from 90.8 to 82.2 mg/dL and 137.4 to 122.4 mg/dL, respectively, after the supplementation, while, in control group there was an increase by 20.1 and 28.6 mg/dL, respectively. The lipid profile data in experimental group reflected that there was 9, 22, and 6.9 mg/dL decrease in serum cholesterol, triglycerides, and LDL cholesterol levels, respectively, while the control group inmates showed a slight increase in serum cholesterol, triglycerides, and LDL cholesterol levels 8.8, 13.4, and 8.7 mg/dL, respectively. Supplementation of fermented beverage showed significant improvement in BMI grades, hemoglobin and reduced blood glucose level, triglycerides, and improved high-density lipoprotein (HDL) cholesterol levels in experimental group when compared with the control group.

Bittergourd juice consumption is traditionally being practiced for the treatment of diabetes mellitus in developing country such as India, but not supported through clinical data and highly bitter juice is difficult to drink. Therefore, bittergourd fermented beverage with improved nutritional strength and taste was developed and supplemented to diabetic subjects and the evaluation was carried out by Devaki and Premavalli (2014b). The evaluation of the beverage was carried out in two stages. In the first stage, 85 subjects were considered in the study and the study was grouped into 3 groups, viz., control-diabetic (30 subjects), experimental-diabetic (30 subjects), and experimental-nondiabetic (25 subjects), and the beverage was supplemented to the experimental group, in the early morning as first drink (45 mL), control group was asked to drink water. Subjects of both experimental and control group were examined for FBS and PPBS in the duration of every 3 and 4 days for a period of 1 month supplementation to observe the impact of the supplementation on sugar profile levels. The impact of supplementation on diabetic subjects, in the first-stage study showed that, reduction of polyphagia by 38%, fatigue by 37%, polyurea by 33%, general weakness by 31%, and burning feet by 19%. In the experimental diabetic subjects, FBS reduced from 213 to 146 mg/dL in males and from 176 to 134 mg/dL in females and PPBS reduced from 263 to 196 mg/dL in males and from 276 to 227 mg/dL in females, respectively, showing 24%–31% of FBS and 18%–25% of PPBS reduction in the experimental group, while in the control group it was observed that reduction of 4 and 15 mg/dL in FBS and PPBS levels, respectively, and in the group of experimental nondiabetic subjects the beverage had more influence on PPBS than FBS. Therefore, even nondiabetic subjects too can consume the developed beverage as a health drink as it influenced more on PPBS by not affecting FBS. In the second stage, the study continued by supplementing the beverage for 16 diabetic subjects with their consent for a period of 5 months. Every week the blood sugar profile was analyzed. FBS reduced from 210 to 120 mg/dL (43%) and PPBS reduced from 283 to 167 mg/dL (41%). Estimated average glucose reduced from 161.05 ± 38.17 to 118.24 ± 25.39 mg/dL. Glycosalated hemoglobin (HbA1c) results indicated that the subjects had fair control of glucose levels before the supplementation, where as after the supplementation they have raised to good control in the glucose levels. The effect of beverage supplementation showed no significant changes in the lipid profile. Therefore, the study clearly reflected the positive effect of bittergourd fermented beverage in reducing and controlling blood sugar levels.

Marimuthu et al. (2015) have studied the cholesterol reduction potential of lactic acid bacteria and *Weissella* strains from gherkins and koozh and these strains exhibited maximum cholesterol reduction with bile salts. Kimchi is claimed to have anticancer, antiobesity, antiaging, and anticonsumption effects while, kefir reduces lactose intolerance symptoms, stimulate the immune system, lower cholesterol, and have antimutagenic and anticarcenogenic properties (Guzel-Seydim et al., 2011). An et al. (2013) had conducted the clinical trials on prediabetic personnel for 8 weeks with the consumption of fermented kimchi and a control group was given fresh kimchi juice. The investigation revealed that fermented juice consumed group had decreased insulin resistance, increased insulin sensitivity, and improved glucose tolerance was 33.3% as against 9.5% in control group. Systolic and diastolic blood pressure decreased significantly in the fermented kimchi group. However, decreased body weight, BMI, and waist circumference were observed in both the groups. Although the microbiota-gut-brain axis is a nascent field of research, there is an indication that fermented food consumption can alter mood and brain activity (Omagari et al., 2014; Tillisch et al., 2014; Hilimire et al., 2015).

The human study on bioavailability of iron from lactic fermented vegetables has been conducted by Nathalie et al. (2016). The increased absorption of iron was found in the subjects in both high and low phytate lactic fermented vegetables and the in vitro study on greater nepcidin response indicated the better availability of iron as compared to fresh samples. The increased bioavailability may be an effect of increase in ferric iron caused by the lactic fermentation.

Wieslaw et al. (2016) have conducted the randomized clinical study in 13 human volunteers by supplementing fresh and fermented cabbage. Blood and urine samples were collected before and after consumption. Fresh cabbage consumption showed higher antioxidant capacity as compared to fermented one. The study showed that the fermentation process affects red cabbage anthocyanin availability and human plasma antioxidant capacity.

Thus, the evaluation of fermented vegetable beverages is being taken up by the researchers in the recent years. Greater emphasis is needed for evaluation of products thereby, the introduction of fermented beverages can see the light of the day under the safety foods.

8.4 Conclusion

The fermented vegetables as well as vegetable beverages are globally gaining popularity thereby development of products has become the target of researchers. Lactic acid bacteria fermentation is the most common method followed and yeasts have its place for development of alcoholic beverages. The fermentation results in nutrients formation, such as vitamins, minerals, lower chain acids, phenolics, antioxidants, etc., improvement of taste and flavor formation which leads to better acceptability and longer shelf life. In vegetable beverages, the studies on nutrients profile as well as the evaluation of products through animal and human studies have received the attention for the past one decade and are quite encouraging in inferring that it provides a greater support for better health of human. The progressive work on these aspects up to 2017 has been covered in this chapter. Considering the reemergence of traditional and regional fermented foods with newer perspectives on the basis of ongoing developments in this field, it is anticipated that fermented beverages will continue to be a growing sector within the functional food market.

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9

FERMENTED MALT BEVERAGES AND THEIR BIOMEDICINAL HEALTH POTENTIAL: CLASSIFICATION, COMPOSITION, PROCESSING, AND BIO-FUNCTIONAL PROPERTIES

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9.1 Introduction

The interest in nonalcoholic fermented beverages has increased in recent years to meet growing consumer demands. Nonalcoholic fermented malt beverages result from the lactic acid fermentation of malted cereal grains, generally barley. These beverages are enjoyed in various parts of the world, including Asia, Europe, Latin America, the Middle East, and South Africa (Taylor and Dewar, 2000; Taylor and Taylor, 2002; Alu'datt et al., 2012; Taylor, 2016). The malting process converts the raw grains into malt; this involves germinating the grains to increase the amount of fermentable sugars, followed by drying, cleaning, and storage. Malting is a key processing step to enhance the degradation process of cell walls through activation of enzymes and softening

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of barley kernels which leads to the production of desirable compounds and enhances the sensory properties (e.g., different flavor, aroma, and color products) of malt products including alcoholic and nonalcoholic malt beverages (Gupta et al., 2010; Nsogning Dongmo et al., 2016). The malting process used in in the production of nonalcoholic beverages includes several steps, namely steeping, germination, and drying under temperature and humidity conditions that enhance the chemical, sensory, and nutritional properties of the fermented products (Nsogning Dongmo et al., 2016; Gupta et al., 2010). Several health benefits of fermented malt products, including alcoholic and nonalcoholic beverages, have been recognized, including antiinflammatory, antidiabetic, antibacterial, antiviral, antithrombotic, antiallergenic, antidiarrhea, and vasodilator actions, beneficial action against certain cancers and gastrointestinal diseases, stimulating lactating glands, as well as strengthening of hair (Cook and Sammon, 1996; Gupta et al., 2010; Mahmoudi et al., 2015; Nsogning Dongmo et al., 2016). The growing consumer demand for health-promoting foods is especially favorable to the development of nonalcoholic malt beverages with no chemical preservatives and additives (Roethenbaugh, 2007). The value of raw cereal (or grain) materials for the nonalcoholic beverage industry has been studied extensively due to their high contents of fiber, high nutritional value, presence of bioactive compounds, low cost, and constant availability throughout the year (Adil et al., 2012; Alvarez-Jubete et al., 2009; Fardet, 2010; Sarwar et al., 2013). Nonalcoholic malt beverages have a superior nutritional value compared to alcoholic beers (Jepsen, 1993; Nsogning Dongmo et al., 2016). Malted barley has been used as juice in the food industry for producing alcoholic and nonalcoholic beverages from malted barley extracts (Briggs, 1978). The phenolic compounds in malted barley grains are well known nonenzymatic inhibitors of lipid peroxidation (Eksiri et al., 2014). Variations in the phenolic content of different varieties of malted barley are thought to play a role in the extension of shelf life of beer and nonalcoholic beverages (Gupta et al., 2010; Nsogning Dongmo et al., 2016). Malt products have various contents and types of endogenous free and bound phenolic compounds. In addition to their antioxidant properties, phenolic compounds can take part in the Maillard reaction, which plays a key role in the development of flavor and aroma of malting and brewing products (Goupy et al., 1999; Nsogning Dongmo et al., 2016). In Munich-style malts, the presence of melanoidins resulting from the Maillard reaction was found to stabilize the beer taste (Briggs, 1998). Several malted drinks have been developed recently to satisfy the consumer demands in various parts of the world (Akpanyung, 2002). Barley grains (seeds) have been used historically and are well known as a primary raw material for beer brewing and production of nonalcoholic beverages (Mackie and Slaughter, 2000). This is due to several unique physiochemical properties that lend themselves well to fermentation and other processing, including their ability to easily germinate and undergo hydrolysis, high content of starch, and moderate content of proteins (Steiner et al., 2012). Additional characteristics make barley a highly valuable grain product for the development of novel and functional beverages and foods.

9.2 Botanical Characteristics of Barley

The barley plant is a short-season and early maturing crop that can adapt to a wide range of environmental conditions compared to other cereals. Cultivars of barley can grow under poor nutritional and harsh soil conditions including cold, drought, saline, and alkaline soils (Ingversen et al., 1973). Barley (*Hordeum vulgare* L.) belongs to the grass family Poaceae (or Gramineae) and the genus *Hordeum* (Von Bothmer, 1992; Lahouar et al., 2011, 2014). Wild barley and cultivated barley were originally the two known species, now more than 32 species are cultivated (Harlan, 1978). Barley has a typical seed like the other members (e.g., rye and wheat) of the Triticeae botanical tribe (Jorgensen, 1982; Holme et al., 2017). The production of tillers (side shoots) helps barley tolerate harsh environmental conditions. Both the head and spike are the portions of the plant that contain the flowers, which subsequently transform to produce mature seeds called kernels or grains (Harlan, 1978; Jadhav et al., 1998). The barley kernel is spindle shaped, length ranging from 7 to 12 mm (Reid, 1985; Jadhav et al., 1998). The barley kernel is composed of three main parts, namely the outer layers (husk, pericarp, testa, and aleurone layers), the endosperm, and the germ or embryo (Poppitt, 2007; Fox, 2010). The husk (or hull) is the protective outer coating of the seed (Reid, 1985; Jadhav et al., 1998). The aleurone layer surrounds the endosperm and contains protein granules in a single layer of cells (Kent and Evers, 1994; Jadhav et al., 1998). The endosperm is the largest part of the kernel; it contains starch granules from which simple sugars (maltose and glucose) are produced to support the growth of the germinating embryo (Jadhav et al., 1998).

9.3 Uses and Applications of Barley Grains

Barley seeds are highly suitable cereal grains for the human diet due to their high contents of B-complex vitamins, fiber and phenolic compounds, and low contents of fat and cholesterol (Ullrich and Shewry, 2014). The uses and applications of barley and its products in the food industry are diverse. They include malting, alcohol production, production of a broad range of malt and malt-based food products (including functional foods and beverages), and ingredients (e.g., coloring and flavor extracts), as well as livestock feeds (Newman and McGuire, 1985; Ullrich and Shewry, 2014). Hulless barley varieties are most popular in the food industry (Tashi, 2005) because little processing is required to remove the outer hull of these varieties. Further processing is used to produce polished or pearled barley, which can be used in cooked food dishes such as soups and salads (Jadhav et al., 1998; Ullrich and Shewry, 2014).

Barley flakes are prepared by steam rolling and drying barley kernels, while barley grits are prepared by cutting barley kernels into small pieces that can be used in processed cereals (Tashi, 2005). Barley can be used instead of rice in many food products. Likewise, the health benefits of barley make it attractive for use as a rice extender (Ikegami et al., 1996). Some commercially available products are a mixture of barley and rice that is prepared by precooking pearled barley and adding to cooked rice (Yang et al., 1999). In addition, malted barley is used in many foods to enhance color, enzyme activity, flavor, sweetness, and nutritional quality (Hansen and Wasdovitch, 2005). For instance, malt ingredients are used in baking industry to improve the dough properties. These include diastatic malt powder, which contains active enzymes that are used little in bread making. Diastatic malt is prepared from sprouting barley which is then dried and grounded into a powder. Malts can be prepared with different degree of color and flavor compounds (Hansen and Wasdovitch, 2005). Knuckles et al. (1997) also reported the use of barley fortification to enhance the quality of baked products. Similarly, the β-glucan fraction obtained from barley seeds was added to pasta to enhance the content of soluble fiber in response to consumer demand (Knuckles and Chiu, 1999). In Asian countries, barley tea is a well-known drink prepared from roasted barley (Pazola and Cieslak, 1979). Elsewhere, barley is added to coffee substitutes to enhance their flavor and overall quality (Pazola and Cieslak, 1979).

9.4 Health Benefits of Barley Grains

When used in fresh and processed foods, barley is a valuable source of several nutrients (mainly, complex carbohydrates including fiber; proteins; unsaturated lipids and phytosterols; vitamin E and vitamins of the B-group; and minerals such as magnesium, potassium, iron, and zinc) and other functional and biological active compounds (e.g., phenolic compounds) (Horowitz et al., 1993). Several chronic diseases, including cardiovascular diseases and diabetes have been studied in relation to barley products and its constituents (Lupton et al., 1994; Ramakrishna et al., 2017; Idehen et al., 2017). Barley extracts have also been used to decrease the symptoms of gastroenteritis and chemotherapy (e.g., nausea and mouth sores) (Wallace, 1930).

β-Glucan, a soluble fiber that belongs to the class of indigestible polysaccharides, is the primary building block of cell walls of barley grains (Hallfrisch et al., 2003). Brand-Miller (1994) showed that β-glucan isolated from barley reduces the glucose and insulin responses in healthy men and women. This property confers a moderate or low glycemic index (GI) to breads, porridge, boiled barley, and other products made from barley (Foster-Powell et al., 2002). High GI foods in contrast cause a rapid increase in blood glucose levels by promoting rapid absorption of simple sugars through the small intestine lining (Jones, 2007). High GI foods tend to increase the resistance of the body to the action of insulin, an undesirable condition known as insulin resistance (IR) (Shimizu et al., 2007). The IR is a known cardiometabolic risk factor frequent in type 2 diabetes and associated with the metabolic syndrome (Meigs et al., 2007). Sato et al. (1990) reported that the blood glucose levels of diabetic patients and healthy people have reduced after the consumption of barley. Indigestible dietary β-glucan from barley grains and the ratio of amylose to amylopectin in barley starch both contribute to the moderate-to-low GI of barley-based foods (Nilsson et al., 2008). The consumption of barley was also found to enhance satiety due to high content of total fiber together with proteins and low-fat content (Pick and Hawrysh, 1998; Nilsson et al., 2008).

β-Glucan from barley grains also plays a vital role in regulating serum cholesterol (Trowell, 1975). This cholesterol-lowering action is related to the increased viscosity of the gastrointestinal tract content when foods rich in β-glucans are consumed (Tietyen et al., 1990). The levels of plasma triglycerides and low-density lipoproteins (LDL) (bad cholesterol) were lowered in people after introducing barley-based foods as a substitute for rice or wheat-based foods (Ikegami et al., 1996). Barley bran flour is considered a hypocholesterolemic ingredient (Goddard et al., 1984; Lupton et al., 1994). Moreover, the fermentation of indigestible β-glucans and resistant starch by microflora in animals and humans produces short- and medium-chain fatty acids, such as butyrate and propionate (Topping and Clifton, 2001), which may have beneficial effects against intestinal and extraintestinal diseases (Canani et al., 2011). Topping et al. (2003) reported that butyrate and propionate enhance the health of the colonic mucosa and act as energy sources for epithelial cells.

9.5 Phenolic Compounds in Barley Grains

Phenolic compounds are secondary metabolites originating from plant sources. Dietary phenolic compounds are increasingly recognized as health-promoting phytochemicals due to antioxidant, antiaging, anticarcinogen, antiinflammation, and antiatherosclerosis properties (Naczk and Shahidi, 2004; Alu'datt et al., 2012; Idehen et al., 2017; Ramakrishna et al., 2017). Han et al. (2007), Idehen et al. (2017), and Ramakrishna et al. (2017) reported that the presence of natural phenolic compounds in foods and their by-products improve the biological properties. In barley and other cereal grains, phenolic compounds are mainly located in the pericarp (Han et al., 2007). In different varieties of barley, the predominant phenolic acids were found to be ferulic acid and *p*-coumaric acid (Holtekjolen et al., 2006). Holtekjolen et al. (2006) further found that the amount of phenolic acids varied according to the occurrence or lack of hull, with significantly higher levels in the hulled varieties. The major antioxidant activity of phenolic compounds in barley grains was related to benzoic acid, cinnamic acid, proanthocyanidins, quinones, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds (Goupy et al., 1999; Bonoli et al., 2004; Alu'datt et al., 2012; Idehen et al., 2017). As in other plants, the phenolic compounds in barley are present in both free and bound forms (Alu'datt et al., 2012). The most abundant free phenolic compound found in barley is proanthocyanidins (or flavonoids), while the predominant bound phenolics are polymers of ferulic acid and its dehydro-dimer derivatives which are ester-linked to the barley cell walls (Hernanz et al., 2001; Alu'datt et al., 2012). It has been estimated that the majority of phenolics (~70%–80%) found in beer come from malted barley, while the remaining (~20%–30%) come from hops (Goupy et al., 1999; Gerhauser, 2005).

9.6 Antioxidant Properties of the Phenolic Compounds in Barley Grains

Antioxidant activity is the ability of bioactive compounds to prevent, delay, and protect against oxidation of various substrates such as DNA and lipid materials, both in living organisms (e.g., humans) and in food products (Shahidi, 2000; Gutteridge and Halliwell, 1994; Naczk and Shahidi, 2004). Phenolic antioxidants can thus play an important role in preventing deterioration of foods and maintaining their quality (Shahidi, 2000). It has been suggested that diets with lower levels of antioxidants could lead to detrimental changes in DNA, lipids, and proteins in human tissues (Papas, 1999). Dietary antioxidants, including phenolics and certain vitamins, decrease the oxidative damage caused by reactive oxygen species (ROS) to DNA, lipids, and proteins, which is thought to be beneficial for preventing and managing various conditions and chronic illnesses (Bendelow, 1975; Naczk and Shahidi, 2004). Velioglu et al. (1998) found that the phenolic constituents of barley grains with the highest antioxidant activity were flavonoids, C-glycosides, saponarin, and lute Orin. Others found that benzoic acid, cinnamic acid, proanthocyanidins, quinones, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds were the major contributors to the antioxidant activity from the phenolic constituents of barley grains (Goupy et al., 1999; Bonoli et al., 2004; Alu'datt et al., 2012; Idehen et al., 2017).

Phenolic compounds can exert a protective antioxidant action through different mechanisms, including reduction of oxygen concentration, termination of free radicals, decomposition of primary products of oxidation to nonradical species, interrupting the continued hydrogen abstraction from substrates, and chelating metal ions (e.g., iron) that promote the formation of highly reactive and detrimental peroxidation compounds (Naczk and Shahidi, 2004). The chemical structures of phenolic compounds allow them to act as antioxidants in preventing the oxidation through donation of a hydrogen atom, thus opposing the formation of free radicals which are detrimental to cell structure and functioning (Shahidi, 2000). Through their action, phenolic compounds seem to support the action of other important antioxidants such as vitamin E (tocopherols) and carotenoids (Skibdsted, 2012). Concerted actions by antioxidants are highly relevant for food stability, human nutrition, and the prevention and management of chronic diseases. Additional modes of action of phenolic compounds, also involving cellular protection but extending beyond antioxidant activity, are becoming recognized. These include modulatory actions in cells through modification of cell-signaling pathways, enzymatic activity, and epigenetic modifications that regulate gene expression (Tsao, 2010).

9.7 General Chemical Composition of Barley Grains

Main components of barley grains are carbohydrates, proteins, lipids, vitamins, minerals, and phytochemicals (Aman and Newman, 1986). On a dry basis, they contain ~70%–82% carbohydrates, 10%–13% proteins, 2%–2.5% lipids, and 2.7% ash (Starling, 1980; Fox, 2010; Ullrich and Shewry, 2014). Barley carbohydrates include dietary fiber (i.e., indigestible carbohydrates) such as β-glucan, cellulose, hemicellulose, lignin, gums, and mucilage, which have several benefits for human health (Nilan and Ullrich, 1993). The chemical composition of barley grains varies depending on plant genotype, agricultural practices as well as environmental conditions (Dehaas and Goering, 1972; Holtekjolen et al., 2006; Fox, 2010; Ullrich and Shewry, 2014).

9.7.1 Barley Carbohydrates

Barley contains two types of carbohydrates, namely complex and simple carbohydrates (Newman and Newman, 2006; Ullrich and

Shewry, 2014). Simple carbohydrates are low molecular weight (MW) monosaccharides and disaccharides, collectively known as sugars. Simple carbohydrates found in barley and barley malt include glucose and fructose (monosaccharides) and sucrose and maltose (disaccharides) (Huang et al., 2015). About 50% of the sugar in barley grains is sucrose; in the endosperm tissue of raw barley grains, maltose represents 0.1%–0.2% of the total sugars (MacLeod, 1952). The contents of maltose and glucose in the endosperm gradually increase during malting (germination), following the enzymatic hydrolysis of starch; the glucose that is released upon starch hydrolysis serves as substrate for subsequent fermentation.

Complex carbohydrates are the main components of unprocessed barley grains, representing about 80% of the total dry matter content (Hofer, 1985; Aman and Newman, 1986; Ullrich and Shewry, 2014). Complex carbohydrates are large MW polysaccharides, such as starch, β-glucans, cellulose, hemicellulose, lignin, pectin, and gums (Bendelow, 1975; Ullrich and Shewry, 2014). Amylose and amylopectin are the major components of soluble barley starch, mainly found in the endosperm (Dehaas and Goering, 1972). Amylopectin is a branched-chain polysaccharide in which α-1,4-D-glucose units are branched through α-1,6-D-glucose linkages. In contrast, amylose is a linear chain of α-1,4-D-glucose units (Banks et al., 1972). Amylopectin in barley confers lower viscosity properties compared to amylose (Banks et al., 1972). Barley starch granules are classified into three categories based on their size and morphology, namely large diameter, irregularly shaped, and small diameter granules (MacGregor and Fincher, 1993). Small diameter starch granules comprise 80%–90% of the total number of starch granules and 10%–15% of the total starch weight, while large granules represent 85%–95% of the total starch weight (Goering et al., 1973).

Nonstarch polysaccharides are the major components of plant cell walls (Bendelow, 1975). Generally, in barley, the most abundant nonstarch polysaccharides are β-glucans, arabinoxylans, and cellulose. Cellulose and lignin are found mainly in the hull (Fincher and Stone, 1986; Duffus and Cochrane, 1993), while β-glucans are present in the aleurone and endosperm tissues (Miller and Fulcher, 1994). Nonstarch polysaccharides that cannot be digested by humans are known as dietary fiber; they are poorly absorbed in the small intestine but exert beneficial physiological and biological actions in humans. Water-soluble dietary fiber found in barley include cellulose and hemicellulose, while insoluble fiber include β-glucans, arabinoxylans, l,4-β-D-glucan (cellulose), glucomannan and 1,3-β-glucan (Aman and Newman, 1986; Ullrich and Shewry, 2014).

9.7.2 Barley Lipids

Lipids are present in relatively small amounts in barley grains and are mostly unsaturated fatty acids. Barley lipids also include phytosterols (Lampi et al., 2004); plant sterols in general have positive health effects and can assist in lowering blood cholesterol. The barley embryo contains about 18% of the total lipid content, while 3%, 5%, and 67% of total lipid contents are found in the endosperm, hull, and aleurone layer, respectively (Price and Parsons, 1974). Some of the lipids from outer layers of the grains are used to produce barley essential oil. Two types of lipids have been identified in barley, namely nonstarch lipids and starch lipids (Morrison, 1993). Nonstarch lipids are located outside the starch granules and exist either in a free form or bound to proteins. In contrast, starch lipids, also known as internal starch lipids, are found inside the starch granules (Tester and Morrison, 1992). The free lipids are extracted with nonpolar solvents, while bound lipids are extracted with polar solvents (Morrison, 1993). The contents of nonpolar lipids and polar lipids (e.g., glycolipids and phospholipids) are 75% and 25%, respectively (Morrison, 1993).

9.7.3 Barley Proteins

The protein content of barley grains, which ranges between 10% and 13%, is influenced by several factors including genetics and growing conditions (Brandt, 1976). The proteins in cereal grains are classified based on their solubility during sequential solvent extractions, which differentiates between four major protein groups, namely albumin, prolamin, globulin, and glutelin (Osborne, 1924). Albumins and globulins are the major soluble protein fractions, being soluble in water and dilute salt solutions, respectively; while prolamins and glutelin are soluble in aqueous alcohol solutions and dilute acid or alkaline solutions, respectively (Shewry, 1993). Barley prolamin is located in the cells of the starchy endosperm and represents 36% of total barley proteins (Kirkman et al., 1982). The albumin and globulin fractions contain structural, metabolic, and protective proteins (Bhatty, 1986). 7S storage globulins are mainly found in the aleurone layer surrounding the endosperm and in the scutellum of the embryo (Entwistle, 1988). Barley contains gluten, which is a mixture of proteins classified as the prolamins and the glutelin, but in a smaller amount than in wheat.

Cereal proteins are further classified into storage proteins and nonstorage proteins. The main storage protein fractions are prolamins, glutelins, and globulins, which comprise between 35 and 50% of the total nitrogen of endosperm proteins in barley (Shewry et al., 1980). Storage proteins form a matrix around the starch granules in the endosperm and provide nitrogen to the growing embryo when germination occurs (Fox, 2010). Nonstorage proteins in contrast are found in the aleurone and embryo, and comprise between 15% and 30% of the total barley proteins (Munck, 1970, 1972). The contents of lysine and threonine (two essential amino acids) are generally greater in nonstorage proteins than in storage proteins (Brandt, 1976; El-Negoumy et al., 1979; Kirkman et al., 1982; Shewry et al., 1984).

Barley prolamins have a poor nutritional quality due to the deficiency of the essential amino acid lysine (Shewry et al., 1984). Prolamins are made of five main protein subunits that contain low amounts of basic amino acids and high amounts of glutamine and proline (Doll and Brown, 1979). Three polypeptide subfractions (A, B and C) have been identified in barley prolamins (Miflin et al., 1983). Polypeptides in subfraction A have a low MW and represent between 1% and 2% of the total prolamin content (Shewry et al., 1978a); polypeptides in subfraction C represent 95% of the total prolamin content (Shewry et al., 1978b). The content of each subfraction was found to vary depending on the stage of maturation of the seed. Specifically, the content of the C subfraction decreased from 33% of the total prolamin content in the early stage of maturation to 15% in the final stage (Rahman et al., 1982). For subfraction B, the content increased from 30% of the total prolamin content during early maturation to 45% at the end of the maturation (Rahman et al., 1982).

Hordein, a prolamin protein, is the major storage protein in barley, representing 40%–50% of the total proteins (Shewry, 1995). Hordein is a mixture of proteins, which can be classified based on their amino acid composition into three major groups, namely sulfur-rich, sulfur-poor, and high-MW prolamins (Shewry and Tatham, 1987). Hordeins have an amino acid composition that is similar to the composition of the prolamin gliadins that are found in wheat, except for the higher contents of proline, lysine, and valine in hordeins (Ewart, 1977). Hordeins may also be classified into four groups, designated as B, C, D, and γ hordeins, based on their electrophoretic mobilities. All the four fractions are soluble in aqueous alcohol solutions (Pomeranz and Ward, 1972). Hordein B is sulfur-rich, hordein C and γ are sulfur-poor, and hordein D has high MW (Shewry et al., 1984). The MW of these fractions range from 53 to 64 kDa (Shewry et al., 1985). There is a relationship between the content of the hordein fractions and the β-amylase activity in grain, which influences grain quality (Ullrich, 2002). Shewry et al. (1980) reported that hordein B contains three subfractions (B_l, MW 35 kDa; B_2, MW 40 kDa; and B_3, MW 46 kDa). For hordein C, two subfractions were identified (C_l, MW 59 kDa and C_2, MW 72 kDa), while for hordein D, the MW was 105 kDa (Miflin et al., 1983).

9.7.4 Amino Acid Composition

The biological quality of dietary proteins refers in part to the quantity of essential amino acids they contain (De Man and Dondeyne, 1985; Sikdar et al., 2016). The contents of proteins in barley is moderate (around 10%) but it has a higher nutritional value compared to other cereals such as legumes, wheat, and their products (Bhatty et al., 1979; Sikdar et al., 2016). As a whole, barley proteins are a good source of the limiting essential amino acids, including lysine, threonine, methionine, and tryptophan (El-Negoumy et al., 1979; Sikdar et al., 2016). Like other nutrients and nonnutrient constituents, the amino acid content of barley grains is influenced by several factors such as genetics, environmental conditions, and soil type (Brandt, 1976). Hofer (1985) found that a high level of nitrogen in soil increases the contents of proteins, essential amino acids as well and nonessential amino acids in barley grains.

9.8 Functional Properties of Barley Proteins

The functionality of food constituents refers to the physical, chemical, and physicochemical properties that affect their technological utilization and function in foods (Kinsella, 1976a,b). This set of properties is distinct from nutritional properties (which are biological properties), but there are relationships between both sets properties. Most of functional properties have an effect on the sensory properties of food ingredients and their products during their preparation, processing, and storage. Several blends of food proteins were added to foods to enhance chemical, functional, nutritional, and sensory properties of foods and their products (Pearce and Kinsella, 1978). Functional properties (e.g., emulsification, foam, gelation, and water-holding capacity) have been used to improve appearance, flavor, texture, and aroma.

Functional protein in food system means the interaction of protein component with other food components, food system, and environment such as composition, structure, and conformation of the proteins, pH, temperature, ionic strength, and food components (Kinsella, 1976a,b). Functional properties are classified into hydration properties, protein-protein interaction, and surface properties (Ramadan, 2000). Hydration properties include protein-water interaction such as solubility, viscosity, and water absorption. Protein-protein interaction is the gelation properties (Vojdani, 1996). Surface properties include emulsification and foaming properties (Vojdani, 1996). The functional properties of proteins are affected by intrinsic factors (size, shape, amino acid composition, charge of proteins, hydrophobicity, and disulfide bond) and extrinsic factors (pH, temperature, ionic strength, and method of extraction) (Zayas, 1997).

9.9 Barley Grains in Fermented Beverage Industries

Fermented foods, juices, alcoholic beverages, and nonalcoholic beverages have been defined as food ingredients attacked by nontoxic and edible microorganisms through the production of enzymes, health benefits of secondary metabolites (peptides, essential oils, alcohol, acids, phenolic, hydrocarbons, and cyclic compounds), and desirable organoleptic aromas (such aroma, flavors, and textures) for consumers (Steinkraus, 1995). The most important of food ingredient and substrate for fermentation are grains and high carbohydrate content food stuffs. The most important fermented microbes with high nutritional aspects, safety issue (nontoxic), and edible microorganisms are lactic acid bacteria (LAB) (Taylor, 2016).

9.9.1 Nutritious Value of Fermented Alcoholic and Nonalcoholic Beverages

Barley foods have been recognized as a fermented food products such as beer and distilled beverages with several health benefits (Celus et al., 2006; Zarnkow et al., 2008, 2010; Nsogning Dongmo et al., 2016) due to high significant contents of phenolics and bioactive compounds such as chalcones, ferulic acids, proanthocyanidins, cinnamic acid, flavonols benzoic acid, flavanones, and flavones (Hernanz et al., 2001; Manach et al., 2004; Kim et al., 2007). Grains of barley are processed to produce malt by using a traditional method (Alazmani, 2015). Malted products traditionally made from barley grain are classified as raw materials for alcoholic and nonalcoholic beverage industries (Mahmoudi et al., 2015).

Malt beverages are divided into nonalcoholic malt beverages and beer-brewing industries that have similar procedure for production from malt raw materials (Rooney, 1969). However, the main differences between nonalcoholic malt beverages and beer-brewing industry are more nutritious and nonalcoholic product (Jepsen, 1993). Okon and Akpanyung (2005) reported that the contents of reducing sugars in commercial malt beverages were 604–944 mg/dL, in addition to acceptable level of vitamins A and C, while the malt beverage had low contents of hydrogen cyanide, iron, protein, phytate, oxalate, and zinc. Briggs (1978) and Chukwurah (1988) reported the production of malt beverages using barley malt, sorghum, and maize.

9.9.2 Malting Industry in Fermented Alcoholic and Nonalcoholic Beverages

In all 75% of barley produced in the world is used for animal feed, while the remaining 25% is used in malting industry including 2% for human food as nonalcoholic malted beverage and beer-brewing drinks (Baik and Ullrich, 2008; Gupta et al., 2010).

Malting barley process is a chemical process used to enhance the nutritional values and bioavailable of nutrients for barley grains through the activation of hydrolytic enzymes that decompose cell walls and kernels (Zarnkow et al., 2008; Nsogning Dongmo et al., 2016). The products of hydrolytic enzymes for barley grains produce low molecular weight compounds, which are responsible for desirable color, aroma, texture, and flavor (Mahmoudi et al., 2015). Malted barley industry includes several steps starting with steeping and then germination and drying malt under controlled humidity with high temperature (Gupta et al., 2010; Mahmoudi et al., 2015). Malt extract is a concentrated syrup from barley composed of 70%–80% sugar (Aghel et al., 2016). According to the alcohol content, malt drinks are categorized as alcoholic (more than 1.2%), low alcoholic (0.5%–1.2%) and nonalcoholic (less than 0.5%); nonalcoholic malt beverages are manufactured in two forms nonfermentative and fermentative (Briggs et al., 2004).

The important step in malted barley industry is germination step, which stimulates the utilization of storage compounds (Mahmoudi et al., 2015). Germination step of malted barley industry is affected by numerous factors such as variety of barley, contents of carbohydrates, nitrogen, sulfur, enzymes, phenolics, lipids, and proteins in barley and germination time (Mahmoudi et al., 2015; Eksiri et al., 2014). Variety of applications for malted barley have been reported in food industry including coffee malt, malto-dextrin, malt concentrate, colorings, flavorings, vinegar, beer brewing, sweeteners, maltose syrup, infant formula, nonalcoholic beverages, alcoholic beverages, and bakery industry (Mahmoudi et al., 2015). Mahmoudi et al. (2015) reported that the malted barley products have been used as drug in human health for several disease such inflammatory, allergenicity, thrombotic disease, hypertension, diabetes, intestinal diseases, lactating glands, diarrhea, and hair loss. Consumption of malted barley products decreases the levels of malondialdehyde and carbonyl in brain and liver due to the presence of antioxidant compounds such as phenolics (Gupta et al., 2010; Qingming et al., 2010). The main bioactive compounds present in malted barley products are phenolic compounds and Maillard products of malting process (Goupy et al., 1999). Malting process reduces the contents of fat and produce free fatty acids through the hydrolysis of lipid by lipoxygenase in lipolysis process (Goupy et al., 1999; Mahmoudi et al., 2015).

Fig. 9.1 illustrates the schematic diagram for industrial process of nonalcoholic, fermented malt beverages.

9.9.3 Malting Process Source of Flavors

The malting process includes numerous steps of enzymatic and chemical reactions that produce malt wort (a rich nutritious medium), which is a substrate for nonalcoholic beverage (Hassani et al., 2014; Steiner et al., 2011; Zarnkow et al., 2008, 2010). Malt wort is a suitable substrate for growth of bacteria due to acidity (pH 5.2–5.6), presence of prebiotics (such as dextrins and glucan), and high content of nutrients (Krüger and Anger, 1990). The outcome of fermentation malt wort by LAB results an excessive amount of organic acids (Rozada-Sanchez et al., 2008). Aroma of nonalcoholic beverages is classified based on sources into two main types depending on the outcome of malt worth and fermentation process. A typical flavor of malt wort process has attractive and desirable sensory properties, which is produced through glycosides hydrolysis and oxidation, Strecker degradation, Maillard

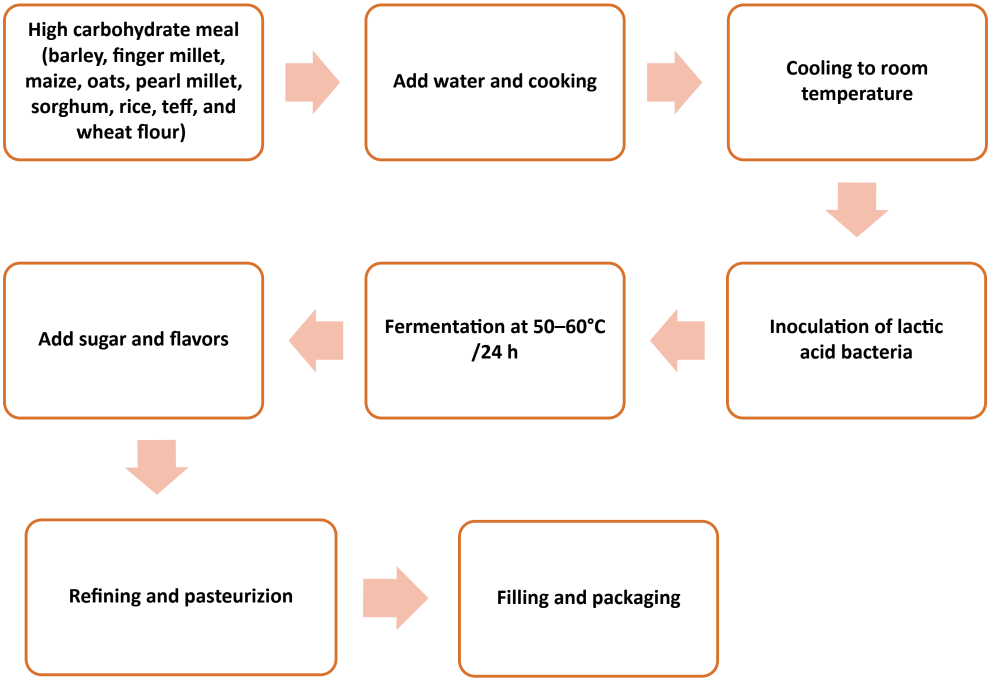
High carbohydrate meal (barley, finger millet, maize, oats, pearl millet, sorghum, rice, teff, and wheat flour)
Add water and cooking
Cooling to room temperature
Inoculation of lactic acid bacteria
Fermentation at 50–60°C /24 h
Add sugar and flavors
Refining and pasteurizion
Filling and packaging

Fig. 9.1 Schematic representation of the industrial process for the production nonalcoholic, fermented malt beverages.

reactions, and fatty acid oxidation (Fickert and Schieberle, 1998; De Schutter et al., 2008a,b). The main objectionable and desirable flavor compounds derived from malt wort and boiling process in nonalcoholic beverages industry are heterocyclic compounds, acids, alcohols, sulfur, aldehydes, furans, esters, lactones, ketones, and terpenoids (Fickert and Schieberle, 1998; De Schutter et al., 2008a,b).

9.9.4 Alcoholic Beverages

Traditional alcoholic beverages have different organoleptic, chemical, nutritional, physical, and sensory properties based on source, cultivar, processing, and consumer needs, for example, in Uganda, the traditional alcoholic beverages do not include the refining process in alcoholic beverage traditional. Several traditional and local alcoholic beverages are produced in Uganda and classified based on raw material sources such as *tonto* from banana, *kweete* from maize and millet, *waragi* from molasses, *ajon* finger millet of *Eleusine coracana*, and *omuramba* from sorghum of *Sorghum vulgare* (Mwesigye and Okurut, 1995). Traditionally prepared beer is a suspended cloudy solution, which is produced by fermentation of cereals by yeast. The main sensory properties are greenish-brown color, milk-like consistency, bitter-sour taste, and musty. Beers have low content of alcohol (≤3%), while the commercial beer is filtered and pasteurized to remove the suspended particles and appear cloudy. The commercial beer is sweetish to sour taste, fruity aroma with alcohol content from 1% to 5% (Taylor and Emmambux, 2008).

Alcoholic beverages are classified based on alcohol contents into three major groups such as wine, beer, and distilled liquors (Dussap and Poughon, 2017). Alcoholic beverages are fermented by yeasts to produce wine and beer, while other types of alcoholic beverages are produced by the fermentation of other microorganisms like fungus *Aspergillus oryzae* (Dussap and Poughon, 2017). The main species of yeast in fermentation process is *Saccharomyces cerevisiae* (Dussap and Poughon, 2017). Distilled alcohols like whiskey are produced by the fermentation of sugars into alcohol and carbon dioxide from different sources of plants such as grapes for wine and barley for beer. The beer is product of fermentation of grains by yeast (*S. cerevisiae*) which ferments only sugar not starch into alcohol. The first step in beer-brewing industry in malting grains through germination of barley seeds, which decomposes starch into sugars through amylase enzyme that may later convert to alcohols. Fermentation of grains produces about only about 3%–6% of alcohol (Dussap and Poughon, 2017).

Removal of alcohol in beer industry is a main key point in sensory profile of beer because beer is a sensitively aromatic beverage. Remove of alcohol (ethanol) from beer may influence on the sensory and organoleptic properties of beers (Brown and Hammond, 2003). Two main techniques were used in nonalcoholic beer industry, physical and biological processes. The physical methods used to remove alcohol completely from beer are vacuum heat, distillation, dialysis, and osmotic distillation (Liguori et al., 2015). While biological methods are used to reduce the production alcohol during fermentation by using special types of yeast strains (Selecky et al., 2008), and continuous fermentation by immobilized yeasts (Pilkington et al., 1998). The sensory properties of the beer produced by biological methods are absolutely different from beer produced by physical methods (Branyik et al., 2012). Beer is an alcoholic beverage with low content of ethanol (1%–10%) which produced by yeasts Saccharomyces, fermentation of barley wort (mixed with maize, wheat, and rice, hop (*Humulus lupulus* L.), and hop extracts. Two main yeast strains are used in fermentation including *Saccharomyces carlsbergensis* and *Saccharomyces vcerevisiae*; the general steps for beer production in brewing industry consist of malting (germination of barley), mashing (malt hydrolysis), separation of nonsoluble hydrolyzed components, boiling of wort (addition of the hop and hop extracts), and fermentation downstream (filtration, stabilization, pasteurization, bottling, etc.). Two stages of fermentation have been reported in beer industry (primary and secondary fermentation) (Linko et al., 1998). Beer is classified as safe drink/beverage, stable, and with longer shelf life due to high contents of hop bitter compounds and carbon dioxide (0.5%), low pH (3.8–4.7), presence of ethanol (1%–10%), low content of oxygen, and low content of nutritive compounds (maltose, glucose and maltotriose) (Jespersen and Jakobsen, 1996; Sakamoto and Konings, 2003; Vaughan et al., 2005).

Brewing industry focus on increasing human safety practices and prevent growth of spoilage microorganisms through refrigeration, pasteurization, filtration, sulfitation, etc. (Vaughan et al., 2005;Douglas et al., 2006). The most spoiled and hazardous bacteria of beer brewing are *Lactobacillus lindneri Lactobacillus brevis, Pediococcus damnosus* (Jespersen and Jakobsen, 1996; Sakamoto and Konings, 2003; March et al., 2005) and some LAB which affect the quality and sensory properties of the final product (Back, 1994; Jespersen and Jakobsen, 1996; Sakamoto and Konings, 2003; March et al., 2005; Vaughan et al., 2005). During fermentation process in beer industry, *Lactobacillus* species produce excess off-odor, turbidity, and acidity (Fernandez and Simpson, 1995; Hartnett et al., 2002; Sakamoto and Konings, 2003). While *Pediococcus* species produce sarcina sickness, turbidity, granular sediments, acidity, and ropiness and haze (Hartnett et al., 2002). The LAB has a significant role in beer-brewing process through the production antimicrobial peptides (bacteriocins) (Cintas et al., 2001; Cleveland et al., 2001; Diep and Nes, 2002; Cotter et al., 2005) and control the number and types of other undesirable microorganisms which may produce objectionable off-flavor and aroma (Lewis, 1998; Vaughan et al., 2005).

9.9.5 Nonalcoholic Malted Beverages

Recently, production of nonalcoholic food beverages with potential health benefits with no additives in beverage industry has been studied extensively to meet consumer demands (Roethenbaugh, 2007; Vartanian et al., 2007). One of the main interests in food industry is to produce nondairy and nonalcoholic functional food beverage for vegetarian and religious group and people with special disease such as lactose intolerance and cardiovascular diseases (Granato et al., 2010a,b; Nsogning Dongmo et al., 2016). The raw material of cereals in nonalcoholic functional beverages have been used due to nutritional value (high contents of fiber, vitamins, protein, bioactive compounds, and secondary metabolites), cheap source, and availability throughout year (Adil et al., 2012; Sarwar et al., 2013). Historically, LAB have been used in nonalcoholic functional beverages in different regions of the world (Hassani et al., 2015; Waters et al., 2013). The fermentation of malted barley by LAB produce nonalcoholic beverages with pH value of 3.5–4.5 such as functional and traditional fermented beverages (Corbo et al., 2014). The main cereals in the production of nonalcoholic functional beverages are quinoa, rye, wheat, rice, oat, amaranth, sorghum, and barley (Nsogning Dongmo et al., 2016). The main problem with nonalcoholic food beverages produced by fermentation of cereals by LAB is that it is not accepted by consumer due to objectionable off-flavor and taste (Azzurra and Paola, 2009).

Objectionable off-flavor of nonalcoholic food beverages from cereals are mainly malty, sweetish, sour, worty-like and cereal-like flavors (Nsogning Dongmo et al., 2016; Salmeron et al., 2015). Fermented nonalcoholic food beverages from cereals, which are produced by *Lactobacillus plantarum* strain, have better sensory attributes as compared to other LAB (Salmeron et al., 2015; Nsogning Dongmo et al., 2016). The main undesirable attributes of sensory properties in fermented nonalcoholic food beverages are quantity of acetone, ethanol, acetaldehyde, and acetoin (Salmeron et al., 2015). Fermented nonalcoholic food beverages are commercially produced and classified as functional beverage due to fortification with nutrients, probiotics, and flavors. Fruit extracts were added to fermented nonalcoholic food beverages in order to overcome the undesirable off-flavor such as Jovita Probiotisch beverage. Mageu is a fermented nonalcoholic food beverage with probiotic strains of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* (Nyanzi et al., 2010). Many nonalcoholic beverage products have been commercialized in food industry by using lactic acid fermentation. Different genera of LAB are responsible in nonalcoholic fermented beverages, which are composed of 11 genera including *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Weissella*, *Leuconostoc*, *Lactosphaera*, *Vagococcus*, *Lactococcus*, *Carnobacterium*, *Pediococcus*, and *Oenococcus*. Lactic acid fermentation is carried out by anaerobic fermentation, break down carbohydrates substrate (such as glucose), and produce organic acids (like lactic acid). Lactic bacteria are classified based on by-products of fermentation into two major groups: homo- and hetero-fermentation groups. The main by-products of homofermentation are lactic acids, while the main products of heterofermentation are lactic acid, carbon dioxide, and ethanol, and desired flavor compounds (like acetaldehyde and diacetyl). The genera of LAB that are responsible for heterofermentation are *Vagococcus*, *Lactococcus*, *Pediococcus* and *Streptococcus*. However, *Lactobacillus*, *Enterococcus*, *Weissella*, *Leuconostoc*, *Lactobacillus*, *Lactosphaera*, *Carnobacterium*, and *Oenococcus* are main homofermented genera of LAB (Taylor and Dewar, 2000; Taylor and Taylor, 2002; Steinkraus, 1995; Steinkraus, 2002; Taylor, 2016). For nonalcoholic beverages, a mixture of microorganisms were cultured in different intrinsic and extrinsic conditions that affect by-products of fermentation including beverage constituents, moisture, pH, redox potential, and temperature.

Zannini et al. (2013) showed that barley malt wort could be fermented using exopolysaccharide-forming *Weissella cibaria* MG1. The exopolysaccharides and oligo-polysaccharides that were produced during fermentation with this LAB can help modulate the organoleptic properties of the final beverage, thus opening interesting possibilities for producing novel, nutritious and functional beverages (Zannini et al., 2013).

Beverages are a food that is significant by its major features from other food range consumed in their liquid form due to their stimulating and quenching properties (Thekororonye and Ngodely, 1995). Fermented beverages are considered essential for the human diet because fermentation is an economical technology, which develops its sensory properties, advances its nutritional value, and preserves the food (Gadaga et al., 1999). Anywhere, cereals were characterized in 2012 and 2013, taking a high care for being a raw material; in nonalcoholic beverages as functional product with high contents of fiber and phenolics (Nsogning Dongmo et al., 2016). Lactic acid fermented malt-based beverages, known by low pH values (3.5–4.5) and distinguished by the nonalcoholic property, are produced by the fermentation of cereals, cereals substrate, or blends of LAB strains (Corbo et al., 2014; Müller-Auffermann et al., 2013). Since many decades the LAB was used in the production of beverages which is extracted from cereals (Hassani et al., 2015; Singh et al., 2013; Waters et al., 2013). Furthermore, yogurt-like cereal functional beverages and traditional cereal fermented beverages are the examples of the available lactic acid fermented cereal beverages (Corbo et al., 2014; Müller-Auffermann et al., 2013). In the fermented beverages, alcohol is removed from the drink by heating, dialysis, and reverse osmosis, these methods are called physical methods, or by biological methods such as heating of the wort, selecting low alcohol producing yeast strain and/or put an end to fermentation (Briggs et al., 2004). Besides, malt production is done by dissolving wort granulates in water, filtrating, adding pure hop aroma, and carbonation, respectively (Kamil, 2003). Antioxidants resulting from malt inhibit oxidative reactions and are capable of recovering oxygen-free radicals (Vanderhaegen et al., 2006). In addition, one of the main characteristics of malt antioxidants to the consumer health is avoidance and deactivation of ROS associated with several diseases like cardiovascular, cancer, and neuronal diseases (Landete, 2013). There are many health benefits of alcoholic malt drink; it plays an important role in protecting against coronary heart diseases, ulcers, and cancers (Bamforth, 2002).

Malted drinks are classified based on contents of alcohol into high alcoholic (≥1.2%), low content of alcohol (0.5%–1.2%), and nonalcoholic beverage (≤0.5%). In food industry, nonalcoholic malted barley beverages are produced by fermented and nonfermented techniques (Briggs et al. 2004). Briggs et al. (2004) reported that the fermented nonalcoholic malted barley beverages is a technique used to remove alcohol from beverage after fermentation by selected yeast strain using physical method such as heating, dialysis, and reverse osmosis for low alcohol.

9.9.6 Benefits of Fermented Alcoholic and Nonalcoholic Beverages

Nonalcoholic fermentation reduces the viscosity and improves the digestibility and palatability of fermented product through the secretion of amylase enzyme, which hydrolyze the starch (source of viscosity in cereals) into dextrin and sugars. The LAB fermentation has little effect on protein contents in fermented products including alcoholic beverages from cereals. Also, the quantity and quality of nonalcoholic fermented beverages from cereals are enhanced through increasing bioavailability of lysine. In terms of bioavailability of micronutrients and phytochemicals, levels of B vitamins and phytate (myo-inositol hexaphosphate) were increased in nonalcoholic fermented beverages. However, the levels of minerals were not affected by fermentation (Taylor and Dewar, 2000; Taylor and Taylor, 2002; Steinkraus, 1995; Steinkraus, 2002; Taylor, 2016).

Consumption of high-content alcoholic beverages has adverse effect on health (Turner et al., 1977). Nonalcoholic fermentation produces antimicrobial agent such bacteriocins (antibacterial peptides) which inhibit different types of pathogens and promote other types of nontoxic microorganisms. In addition, it may equilibrate the number of microbes inside gastrointestinal tract (Taylor and Dewar, 2000; Taylor and Taylor, 2002; Taylor, 2016). Traditional nonalcoholic fermentation of cereal can be classified based on percentage of water contents into fermented flour, pancakes, doughs, cakes, thick porridges, thin porridges, and beverages (Taylor and Taylor, 2002; Taylor, 2016). Such nonalcoholic fermented products are consumed in different regions of the world including Europe, Asia, Africa, and Latin America (Taylor and Taylor, 2002; Taylor, 2016).

Nonalcoholic fermented beverages have been prepared from barley, finger millet, maize, oats, pearl millet, sorghum, rice, teff, and wheat flour. Different names were used for nonalcoholic fermented beverage brands in Africa (like Hulu-mur in Sudan, Kunun-zaki, in Nigeria, Mageu, mahewu and magou in Southern Africa, Motoho oa mabela in Southern Africa, Munkoyo in Zambia and Oshikundu in Namibia), Latin America (such as Agua-agria in Mexico) and in Europe, Middle East and Asia (e.g., Boza in Bulgaria and Turkey) (Taylor, 2016). Fermented LAB in beverages play vital roles in beer brewing through the inhibition of objectionable spoiled microorganisms which produce undesirable objectionable off-flavor and undesirable acids (Vaughan et al., 2005).

Additionally, LAB in brewing industry may produce food preservative bacteriocins (Cotter et al., 2005) which are peptides with antimicrobial properties for foodborne pathogens and Gram-positive bacteria (Cintas et al., 2001; Franz et al., 2007; Nes et al., 2007; Drider et al., 2006).

Several studies have reported about the potential application of bacteriocins in deactivation and preservation of spoilage microorganism in alcoholic beverages (Nauth, 2002; Vaughan et al., 2005). Hartnett et al. (2002) and Vaughan et al. (2005) reported that the antimicrobial activity of the fermented barley isolated by *Lactococcus lactis* enables long shelf life and stable beer. The main applications of bacteriocins in alcoholic beverages are to increase the shelf life and to produce stable product during pasteurization and filtration (Vaughan et al., 2005). Bacteriocins do not inhibit yeast and it can be added during different stages of production including nonfermented wort, pasteurization, and packaged stages (Vaughan et al., 2005). Bacteriocins inhibit the growth of bacteria that affect the viability of yeast (Vaughan et al., 2005).

Mahewu is a fermented nonalcoholic food beverage fortified with iron (Salvador, 2015). Obiolor is a fermented nonalcoholic food beverage with nutraceutical and pharmaceutical properties (Ajiboye et al., 2016). Grainfields classified as fermented nonalcoholic food beverage with high contents of essential amino acid and vitamins, probiotics, and enzymes (Nsogning Dongmo et al., 2016).

The health benefits of fermented nonalcoholic food beverage are due to low cholesterol, high fiber, gluten-free and high phenolics contents. Products of fermented nonalcoholic food beverage were used by people with cardiovascular diseases to reduce the level of cholesterol and for vegetarianism and veganism people (Nsogning Dongmo et al., 2016). Malt-derived substrates are the appropriate substrates for the growth of LAB and precursor for aromatic compounds (Gebremariam et al., 2015; Salmeron et al., 2014, 2015; Nsogning Dongmo et al., 2016). The brewing process of malting involves numerous steps of nonenzymatic and enzymatic reactions, which produce malt wort. A malt wort is a raw material for functional nonalcoholic food beverage (Zarnkow et al., 2008, 2010; Nsogning Dongmo et al., 2016) that is a good source of aroma precursors through fermentation including amino acids, sugars, phenolic acids, glycosides, and fatty acids (Nsogning Dongmo et al., 2016). Typical aroma of malt wort is vital for the production of alcoholic food. Processed food beverage produced from malted cereal substrates had a superior sensory attributes as compared to unmalted (Nsogning Dongmo et al., 2016). The main source of aromatic compounds in nonalcoholic food beverages are derived from sugar-protein interaction in Maillard reaction, sugar-sugar interactions in Strecker degradation, oxidation in fatty acid oxidation, hydrolysis reactions in glycosides hydrolysis, and oxidation (Nsogning Dongmo et al., 2016). In all 95 aromatic compounds in nonalcoholic food beverages are derived from malt, malt boiling, and wort boiling process such as alcohols lactones, sulfur, heterocyclic, nitrogenous, aldehydes, furans, ketones, esters, acids, terpenoids, and cyclic compounds (Fickert and Schieberle, 1998; Nsogning Dongmo et al., 2016). Aromatic compounds derived from lactic acid fermentation of cereal substrates (including raw material, malt, and malt wort) occur through free fatty acids, carbohydrates, proteins, and free amino acids which produce 74 aromatic compounds such as organic acids, furans, ketones, esters, aldehydes, fatty acids, phenolics, and alcohols (Nsogning Dongmo et al., 2016). The LAB have been used in the production of nonalcoholic malted beverages with low pH (3.5–4.5) (Corbo et al., 2014; Müller-Auffermann et al., 2013) from rye, wheat, barley, amaranth sorghum, quinoa, rice, and oat (Coda et al., 2011; Gebremariam et al., 2015; Kedia et al., 2007; Salmeron et al., 2014, 2015; Zannini et al., 2013). Fermentation of malt wort is key for metabolic activity of LAB and production of aromatic compounds (Gebremariam et al., 2015; Kedia et al., 2007).

9.10 Conclusion

Fermented beverages are considered essential for the human diet because of nutritional, nutraceutical, and pharmaceutical properties. Functional nonalcoholic fermented beverages from barley have been studied extensively due to their possible health benefits related to high contents of phenolic compounds, bioactive peptides, and fibers. Fermented beverages from barley, malt beverage, are classified based on contents of alcoholic into low and nonalcoholic beverages. Nonalcoholic beverages are manufactured in two forms: nonfermented and fermented beverages. The main features for fermented malt beverage are lactic acid contents and pH value (3.5–4.5) which is distinguished by the nonalcoholic property and produced by the fermentation of barley by LAB. Alcoholic fermented malt beverages are removed from the drink by physical (like heating, dialysis and osmotic technique) and biological methods (e.g., heating of the wort, low alcohol producing yeast strain, and inactivation of fermentation methods). Malt processing is performed through dissolving granulates of wort in water followed by filtration, addition of pure hop aroma, and carbonation, respectively. The main characteristics of fermented malt beverages are antioxidants and biomedicinal food/ (such as cardiovascular, ulcers, cancer, and neuronal diseases).

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10

KOMBUCHA TEA: A PROMISING FERMENTED FUNCTIONAL BEVERAGE

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10.1 Introduction

Kombucha tea is manufactured by fermenting sweetened black or green tea with powerful symbiosis of acetic acid bacteria (AAB) and yeasts forming cellulose-like pellicle (zoogleal mat) on its surface (Srihari et al., 2013a). It is a traditional beverage emerged in China several thousand years ago (Cetojevic-Simin et al., 2008). It was first consumed in Asia for its health-promoting benefits. The medicus Kombu used this beverage to treat the digestive troubles of the Japanese Emperor. With the expansion of commerce, the consumption of kombucha expanded to Russia and Germany. In the 1950s its consumption became very popular in France. The habitude of consuming kombucha was palatable throughout Europe till Second World War which caused extensive scarcity of the tea and sugar. According to the researchers in Switzerland, kombucha was similarly beneficial as yogurt (Jayabalan et al., 2014). Kombucha becomes popular in the United States, due to its revitalizing and therapeutic effects. This beverage has been intensely used for a long time worldwide for its prophylactical and curative features (Battikh et al., 2012) with regular consumption, reportedly leads to longevity because of the reversal of aging processes (Kurtzman et al., 2001). This beverage is produced in large scale for commercial use as well as for domestic consumption. Nowadays, kombucha with different flavors is sold worldwide in markets (Dufresne and Farnworth, 2000; Watawana et al., 2015a).

The flavor of kombucha tea is considered to be satisfactory and acerbic, mildly sour and mildly alcoholic, similar to apple cider. As the kombucha fermentation progresses, the taste of the beverage evolves from likeable fruity, sour, and effervescent flavor to strong vinegary taste, thus increasing the consumer acceptability of the flavor and other sensory aspects of the beverage (Goh et al., 2012; Watawana et al., 2015a).

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10.2 Microbial Community of Kombucha Tea

Kombucha is typically produced by inoculation of the tea fungus into a sweetened tea infusion with sucrose for fermentation (Cetojevic-Simin et al., 2008; Battikh et al., 2012). The name of tea fungus is faultily entitled depending on the capability of AAB to form a supernatant bio-cellulose network that looks like surface fungus on the stationary broth (Jayabalan et al., 2014). The microbial composition of kombucha tea culture varies from one culture to another depending on determinants such as geographic location, environment condition, the local species of bacteria and yeast, and the source of the inoculum (Jayabalan et al., 2010). Kombucha tea culture is a symbiosis of AAB (*Acetobacter aceti, Acetobacter pasteurianus, Acetobacter xylinum, Acetobacter xylinoides, Acetobacter peroxydans, Gluconobacter oxidans, Gluconobacter europaeus, Gluconobacter saccharivorans*) and yeasts (*Saccharomyce cerevisiae, Saccharomyce ludwigii, Zygosaccharomyces bailii, Kloeckera apiculata, Schizosaccharomyces pombe, Brettanomyces bruxellensis, Brettanomyce lambicus, Brettanomyces custersii, Torulaspora delbrueckii, Candida stellata, Candida stellimalicola, Candida tropicalis, Candida parapsilosis, Lachancea thermotolerans, Lachancea fermentati, Lachancea kluyveri, Torulopsis* spp., *Pichia mexicana, Eremothecium cymbalariae, Eremothecium ashbyii, Kluyveromyces marxianus, Debaryomyces hansenii, Meyerozyma caribbica, Meyerozyma guilliermondii, Hanseniaspora uvarum, Hanseniaspora meyeri, Hanseniaspora vineae, Zygowilliopsis californica, Saccharomycopsis fibuligera, Merimbla ingelheimense, Sporopachydermia lactativora, Kazachstania telluris, Kazachstania exigua, Starmera amethionina, Starmera caribae*) (Teoh et al., 2004; Malbasa et al., 2011; Chakravorty et al., 2016; Coton et al., 2017).

Lactic acid bacteria (LAB) have also been isolated from some kombucha associations (Marsh et al., 2014; Hrnjez et al., 2014; Ayed et al., 2017; Coton et al., 2017). Marsh et al. (2014) suggested that *Lactobacilli* are more predominant in kombucha, particularly at the later stages of fermentation. They determined *Lactobacillus kefiranofaciens* subsp. *kefirgranum* as the more abundant genera of LAB in kombucha culture. Petrušic et al. (2011) also showed the presence of *Lactobacillus plantarum*, *Streptococcus thermophilus, Streptococcus bovis*, *Streptococcus lutetiensis*, *Brevibacterium* sp. as constituents in kombucha inoculums on black tea. While osmotolerant species started fermentation, the acid-tolerant species dominated finally (Teoh et al., 2004). It has been reported in some studies that the usage of different starter cultures could cause a development of different antioxidant activity pathways although the same substrate has been used for kombucha (Malbasa et al., 2011). The bacteria and yeast numbers reach 10^4–10^6 cfu/mL in a culture which has been allowed to kombucha fermentation for a period of ~10 days. According to many researches, yeast outnumbers the bacterial count (Chen and Liu, 2000; Goh et al., 2012). Kombucha comprised two parts (Fig. 10.1): a supernatant bio-cellulose pellicule segment and the liquid broth (Chen and Liu, 2000). Cellulose floating nets are produced by AAB on the surface where bacteria and yeasts are attached. Bacteria and yeast form a strong symbiosis that can prevent the growth of contaminating bacteria (Jayabalan et al., 2010). The yeasts produce ethanol from sucrose. Glucose and fructose are converted to gluconic acid and acetic acid respectively by AAB. Yeasts are stimulated to produce ethanol by acetic acid. Ethanol, in turn, facilitates growth of AAB and their acetic acid production. Both ethanol and acetic acid are antimicrobials against pathogens (Sievers et al., 1995; Liu et al., 1996). After the increase in the population of LAB and acids, pH of kombucha decreases (Sievers et al., 1995). The LAB could also exert immune-stimulator effects on the host (Marteau and Rambaud, 1993). The tea in the substrate provides microorganisms with required nitrogen compounds abundant in tea (Cetojevic-Simin et al., 2012). Caffeine and related xanthines found in tea enable to stimulate the cellulose synthesis by the bacteria (Dufresne and Farnworth, 2000).

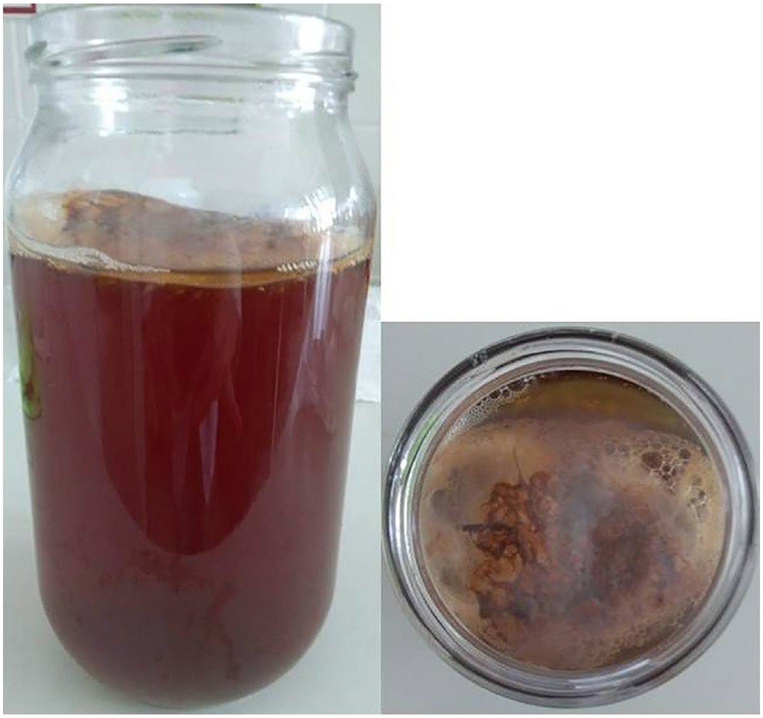


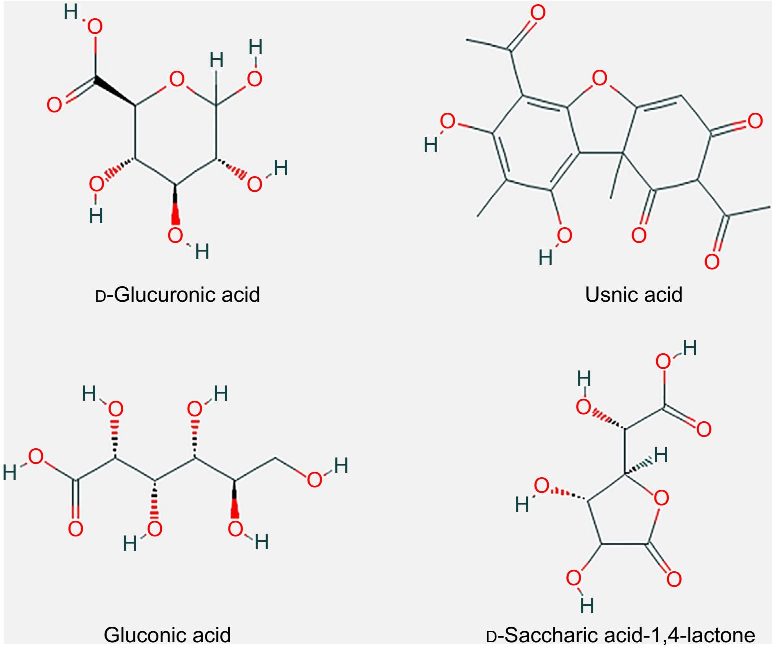
Fig. 10.1 Kombucha black tea having fermented broth and bio-cellulose pellicule segment.

Nguyen et al. (2015a) determined the effects of LAB from different sources such as pickled cabbage, kombucha, and kefir on glucuronic acid production of kombucha and its antibacterial and antioxidant activities. They reported that LAB strains isolated from kefir are outstanding for enhancing glucuronic acid production of kombucha and improving the antioxidant and antibacterial activities of the beverage. Their research demonstrated that *Lactobacillus casei* and *L. plantarum* isolated from kefir has potential as bio supplements for improving the bioactivity of kombucha.

10.3 Physicochemical Composition of Kombucha Tea

Studies on kombucha point out the availability of several organic acids (i.e., acetic, gluconic, glucuronic, usnic, lactic, citric, malic, succinic, pyruvic, tartaric, malonic, and oxalic); various carbohydrates (i.e., glucose, fructose, and sucrose); folic acid, B1, B2, B6, B12, and C vitamins; amino acids, proteins, biogenic amines, purines, lipids, pigments, enzymes (i.e., invertase and amylase), ethanol, carbon dioxide, antibiotically active matters, polyphenols, as well as some tea components (i.e., catechins, theaflavins, thearubigins, and flavonols), minerals, D-saccharic acid-1,4-lactone (DSL), along with inadequately identified metabolites of yeasts and bacteria. Other components present in kombucha beverage are aldehydes, ketones, esters, and ethyl gluconate (Jayabalan et al., 2014; Velicanski et al., 2014). Kumar et al. (2008) reported that kombucha tea includes F, Cl, Br, I, phosphate, sulfate, and nitrate. Chemical structure of some kombucha constituents is depicted in Fig. 10.2. Kombucha culture converts this substrate into a revitalizing beverage with high nutritional and therapeutical features (Lončar et al., 2000). The popularity of kombucha expanded owing to its functional impacts on health and its convenience in domestic production. The quantity of tea, sucrose, and kombucha culture could be different. The results given in Table 10.1 indicate some important components of kombucha tea.

Chakravorty et al. (2016) investigated the dynamics of the microbiota along with the biochemical features of kombucha tea up to 3 weeks of fermentation. The yeast population of the cellulosic biofilm did not change over time and was dominated by *Candida* sp. However, the broth showed a significant shift in dominance from *Candida* sp. to *Lachancea* sp. on the seventh day of fermentation. They reported that *Candida* was the most dominating yeast genus during fermentation. *Komagateibacter* was the single largest genus that existed in both the biofilm and the broth. The bacterial diversity was higher in the beverage than in the biofilm with a peak on the seventh day of fermentation.



D-Glucuronic acid

Usnic acid

Gluconic acid

D-Saccharic acid-1,4-lactone

Fig. 10.2 Chemical structure of some Kombucha constituents. From PubChem, 2017. https://pubchem.ncbi.nlm.nih.gov/.

The biochemical characteristics altered with the progression of the fermentation, that is, the radical scavenging ability increased significantly on the seventh day. The ethanol content increased initially with time reaching up to 0.28 g/L on the seventh day, followed by a decrease to about 0.073 g/L after 21 days of fermentation. This reduction in ethanol depends on its utilization by AAB to produce acetic acid. A significantly higher DSL content reached to 2.24 ± 0.1 g/L after 21 days. Unfermented black tea and also kombucha tea exhibited antiglycation properties. However, the antiglycation properties of black tea were augmented after fermentation by about 25.5%. Thus the results demonstrated that the microbiota of kombucha tea might play significant role in enhancing the antidiabetic property of kombucha. The most recently claimed antidiabetic properties of kombucha may as well be dedicated to its antiglycation activity (Aloulou et al., 2012; Bhattacharya et al., 2013).

Neffe-Skocińska et al. (2017) evaluated the effect of fermentation conditions on physicochemical, microbiological, and sensory properties of kombucha tea beverages. Fermentation was continued for 10 days at 20°C, 25°C, and 30°C. The optimal fermentation conditions of the beverages were a temperature of 25°C and a period of 10 days which enabled to retrieve a product with good physicochemical,

Table 10.1 Dominant Components of Kombucha Tea

Components	Component Content (g/L)	Sugar Content (%)	Black Tea Content	Temperature (°C)	Fermentation Period (day)	References
Acetic acid	8.00	10	Two bags	24 ± 3	60	Chen and Liu (2000)
	6.17 ± 0.13	10	12 g/L	24 ± 3	15	Jayabalan et al. (2007)
	16.57 ± 0.9	10	4.2 g/L	28 ± 2	21	Chakravorty et al. (2016)
	12.53 ± 0.6	10	4.2 g/L	Room temp.	14	Bhattacharya et al. (2013)
Glucuronic acid	1.71	10	12 g/L	24 ± 3	18	Jayabalan et al. (2007)
	3.39	10	1.5 g/L	28	21	Lončar et al. (2000)
Gluconic acid	7.36 ± 0.87	10	4.2 g/L	28 ± 2	21	Chakravorty et al. (2016)
	6.38 ± 0.3	10	4.2 g/L	Room temp.	14	Bhattacharya et al. (2013)
DSL	2.24 ± 0.1	10	4.2 g/L	28 ± 2	21	Chakravorty et al. (2016)
	1.34 ± 0.06	10	4.2 g/L	Room temp.	14	Bhattacharya et al. (2013)

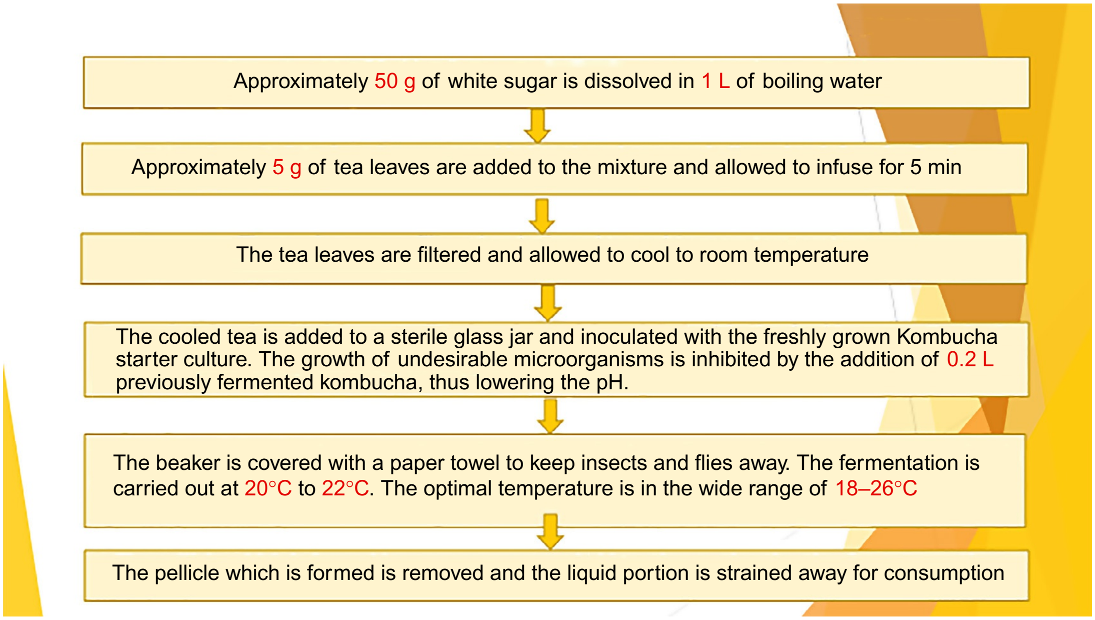
microbiological, and organoleptic qualities. Glucuronic acid content increased during fermentation at all temperatures reaching the highest on the 10th day of fermentation at 25°C. It was observed that the overall quality of all the samples were good, whereas kombucha fermented at 25°C was evaluated as the best.

A part of the biofilm produced during kombucha fermentation is used as starter culture, while the remaining thrown as a waste. The waste biomass has good quality of protein and is also rich in cellulose and hemicellulose. Dried biomass has been successfully evaluated as a protein source in poultry feed (Jayabalan et al., 2010). Bacterial cellulose membrane, a side-stream product of kombucha, was found to be a valuable raw material for bacterial nanocellulose manufacturing. A facile route to obtain high-valued cellulose nano- and microfibrils by purification of kombucha membranes and further mechanical disintegration of the cellulosic macrostructure into nanofibrils is shown in the research of Dima et al. (2017). They presented the results of three different processes of bacterial nanocellulose obtained from kombucha membranes (by microfluidization, atomization, and colloidal milling), the evaluation of the purification degree, the size and aspect of cellulose fibrils after each treatment step, and the physico-chemical properties of intermediary and final products.

10.4 Kombucha Preparation

The kombucha preparation method is as follows (Fig. 10.3).

Potable water (1 L) is boiled and sucrose (50 g) is dissolved in it. Black tea (5 g) is infused for 5 min and then filtered. After cooling down to room temperature, tea broth is poured into a glass jar priorly sterilized with boiling water and inoculated with the culture (24 g). pH is lowered by the inoculation of previously fermented kombucha (0.2 L) and/or cellulosic pellicle (2.5%–5.0%), so the growth of undesirable microorganisms is inhibited. To keep insects away, the jar is covered with a paper tissue and incubated at room temperature. The optimum fermentation temperature is in the range of 18–26°C. The newly formed daughter culture forms a clear thin pellicle and float. There will be gas bubbles due to production of carbon dioxide. The mother culture precipitates to the bottom of the broth. After filtering, beverage is stored in capped bottles at 4°C (Kallel et al., 2012). Approximately 7–12 days of fermentation is necessary for gustable flavor and taste.



Approximately 50 g of white sugar is dissolved in 1 L of boiling water

Approximately 5 g of tea leaves are added to the mixture and allowed to infuse for 5 min

The tea leaves are filtered and allowed to cool to room temperature

The cooled tea is added to a sterile glass jar and inoculated with the freshly grown Kombucha starter culture. The growth of undesirable microorganisms is inhibited by the addition of 0.2 L previously fermented kombucha, thus lowering the pH.

The beaker is covered with a paper towel to keep insects and flies away. The fermentation is carried out at 20°C to 22°C. The optimal temperature is in the wide range of 18–26°C

The pellicle which is formed is removed and the liquid portion is strained away for consumption

Fig. 10.3 The typical method of preparation of the Kombucha beverage using sugared black tea.

Long-term fermentation results in high amounts of acids that may create potential health risks (Sreeramulu et al., 2000; Kallel et al., 2012; Jayabalan et al., 2014).

10.5 Health Benefits of Kombucha Tea

The admitted health benefits related with the consumption of kombucha have been largely referred to the polyphenols of kombucha that have been transformed from black tea. Tea containing theaflavins, thearubigins, theobromine, theophylline, catechins, catechin gallates gallic acid, tannins, gallotannin, caffeine, adenine, small amounts of aminophylline, and volatile oil has strong aroma and taste (Jayabalan et al., 2008). The protective effects of tea polyphenols against various diseases were studied (Yang et al., 2002; Liu et al., 2017). Brewed tea also contains significant amounts of the catechins and flavonoids. The catechins and gallic acid complexes present in the kombucha (i.e., theaflavins, theaflavinic acids, thearubigins, or theasinensis) have a significant degree of bioavailability, as it is prepared from the black tea (Srihari et al., 2013a).

In addition organic acids, vitamins, amino acids, antibiotically active matters, and several micronutrients produced during fermentation of kombucha may also have a role in the health benefits to some extent (Vijayaraghavan et al., 2000; Srihari et al., 2013a). Kombucha has been alleged to be a prophylactic agent and beneficial to health (Liu et al., 1996). Dufresne and Farnworth (2000) reported health effects of kombucha from consumers' declaration and researchers from Russia (Table 10.2).

Also, kombucha has hypoglycemic and antilipidemic properties and radical scavenging activities (Chen and Liu, 2000; Yang et al., 2009; Malbasa et al., 2011; Aloulou et al., 2012; Ilicic et al., 2012; Srihari et al., 2013a; Jayabalan et al., 2014; Watawana et al., 2015a). Moreover, kombucha is accepted as a natural antibiotic and help maintain acid-alkaline equilibrium of the body (Sievers et al., 1995; Cetojevic-Simin et al., 2012; Jayabalan et al., 2014). Extensive study on kombucha tea's ability to prevent gastric mucosal injury and to inhibit progression of gastric ulcers has been carried out (Banerjee et al., 2010). Nevertheless, health benefits were investigated in experimental models only and scientific evidence based on human models is deficient (Jayabalan et al., 2014). Glucose absorption is retarded through the inhibition of brush border enzymes by the inclusion of starch hydrolase inhibitors into the diet. Inhibitors of these enzymes such as α-amylase and α-glucosidase could retard starch digestion, reduce the rate of glucose absorption into the blood circulation, and eventually blunt postprandial plasma glucose rise in diabetics. Fermentation increased α-amylase inhibitory activity (Koh et al., 2009). Oral intake of kombucha

Table 10.2 Health Effects of Kombucha From Consumers' Declaration and Researchers From Russia

- Strengthen general metabolism
- Protects from cancer and cardiovascular diseases
- Enhances the immunity and stimulates interferon production
- Detoxifies blood
- Promotes liver functions
- Balances intestinal flora
- Normalizes bowel activity
- Stimulates glandular systems
- Aids digestion, acts as a laxative
- Regulates cell proliferation
- Provides relief against hemorrhoids
- Improves hair, derm and fingernail health
- Improves visual efficiency
- Reduces cholesterol level, atherosclerosis, blood pressure
- Counteracts aging
- Alleviates arthritis, rheumatism, and gout symptoms
- Reduces inflammations
- Reduces obesity and regulate appetite
- Protects against diabetes
- Prevents microbial infections
- Reduces stress and nervous and sleep disturbances
- Prevents the formation of bladder infections
- Reduces nephrocalcinosis
- Reduces menstrual and menopausal complaints
- Relieves asthma and bronchitis
- Relieves headaches

(5 mg/kg of body weight) in alloxan-induced diabetic rats showed better inhibition of α-amylase and lipase activity in the plasma and pancreas and also better suppression of increased blood glucose levels (Aloulou et al., 2012).

The results of the research of Bhattacharya et al. (2013) showed antidiabetic potential of kombucha as it effectively healed alloxan-induced pathophysiological changes. Besides, it could ameliorate DNA fragmentation and caspase-3 activation in the pancreatic tissue of diabetic rats.

Srihari et al. (2013a) reported the antihyperglycemic effect of kombucha tea in streptozotocin (STZ)-induced rats. They determined that kombucha supplementation (6 mg/kg bw after 45 days) significantly reduced glycosylated hemoglobin (HbA1c) and increased the levels of plasma insulin, hemoglobin, and tissue glycogen which were decreased up on STZ treatment and also significantly reversed the changed activities of glucose-6-phosphatase, fructose-1,6-bisphosphatase, and hexokinase. Their results indicated that kombucha could be esteemed as a probable candidate for future applications as supplement for the prevention and the treatment of diabetes and its secondary complications.

The beneficial effects of kombucha are based on the presence of the metabolic products released into the broth in the course of the fermentation, although most of the health benefits are hypothesized to be due to its radical scavenging activity. The kombucha SCOBY (Symbiotic Culture of Bacteria and Yeast) has the ability to enhance the radical scavenging potential of black tea by fermentation process (Dufresne and Farnworth, 2000). Moreover, the presence of glucuronic acid brings forth the beneficial properties to this beverage (Teoh et al., 2004). Glucuronic acid, an important detoxicant, is a highly polar carboxylic acid and ordinarily produced by liver (Yavari et al., 2011; Vína et al., 2013). The conjugation of glucuronic acid with unwanted compounds increases their solubility and enables their transport and elimination from the body (Vína et al., 2013). This acid can be transformed into glucosamine and chondroitin-sulfate, other acidic mucopolysaccharides and glycoproteins, which are related with collagen and also the fluid that acts as lubricate in the joints (Yavari et al., 2011).

Nguyen et al. (2015b) used both *Dekkera bruxellensis* and *Gluconacetobacter intermedius* as a symbiosis model and investigated glucuronic acid formation of these microorganisms. The inoculation of *L. casei* and variations in sucrose level, temperature, and duration of fermentation were investigated to determine their effects on glucuronic acid production. This model consisted of *D. bruxellensis, G. intermedius,* and *L. casei* with a corresponding ratio (1:1.5:0.5) producing the maximum glucuronic acid (238.60 ± 6.45 mg/L). *L. casei* acted as a supporter species and incited glucuronic acid production to levels 54.1% higher than in the control.

For detoxication and elimination of xenobiotics, support of fat-soluble endobiotics' normal metabolism and prevention of cardiovascular, neurodegenerative, chronic respiratory and kidney diseases, diabetes mellitus type 2 and cancer, consumption of kombucha containing glucuronic acid is recommended (Vina et al., 2013).

Danielian (2005) reported that kombucha tea has curative properties against gout and rheumatism, explained by its detoxifying ability. Butyric acid produced by SCOBY during fermentation is known to protect cellular membranes. In combination with glucuronic acid, this complex enables to strengthen the walls of intestines and give protection against parasites (Dufresne and Farnworth, 2000). The results of the research of Lobo and Shenoy (2015) showed that kombucha has important antihypertrophic, antihyperglycemic, antihyperlipidemic, and membrane stabilizing features. For this reason, kombucha can be advised for patients suffering from cardiovascular diseases as well as for healthy persons every day. Organic acids found in kombucha tea, that is, gluconic acid and ascorbic acid, reduce trivalent iron compounds to divalent iron ions, and form with them chelate compounds that dissolve well in an acidic medium. Such an acidic medium is temporarily formed by the consumption of kombucha tea, providing body with iron. It has been proven that ascorbic acid, present in kombucha tea, also improves iron absorption and is important for preventing megaloblastic anemia in infants (Halberg and Hulthen, 2000). Consumption of kombucha tea is especially advised for geriatric people and vegetarians. Because, it improves the absorption of iron from plant sources and assists in the prevention of iron deficiency (Vina et al., 2014). Also, regular consumption of kombucha contributes significantly to weight gain inhibition and life extension (Hartmann et al., 2000; Battikh et al., 2012).

Gharib (2014) investigated the effect of kombucha (KT) on some minerals (i.e., Fe, Cu, Zn, and Cu/Zn ratio) in brain, spleen and intestine of rats exposed to electromagnetic field (EMF) (950 MHz) originating from mobile phone for 2 months. Control, EMF group, KT group, and KT + EMF group were formed including six randomly chosen rats in each group. The group of rats subjected to EM waves had significant increases in Fe, Cu levels and Cu/Zn ratio accompanied with a decrease in Zn level in brain, spleen, and intestine. Combined consumption of kombucha with EMF resulted in a prospering alleviation of these side effects of EMF. Administration of kombucha to rats exposed to the EM waves showed amelioration in the studied trace element homeostasis which may be due to the adsorption effects of biocellulose pellicle produced by the kombucha culture. Besides, reduced glutathione helps in the reduction of heavy metal ions by accepting electrons. In conclusion, the administration of kombucha to rats exposed to EMW could achieve trace element homeostasis in different organs.

10.6 Antioxidant Properties of Kombucha Tea

Antioxidant potential of kombucha tea is related with its many affirmed positive health effects like prevention of cancer, immunity improvement, relief from arthritis pain, and inflammation. Jayabalan et al. (2008) informed that although total phenolics and free radical scavenging activities were enhanced with prolonged fermentation, reducing power, hydroxyl radical scavenging ability, and antilipid peroxidation ability were reduced. Malbasa et al. (2011) investigated the effects of different cultures on the antioxidant capacity of kombucha prepared by black and green tea. They determined the maximum antioxidant capacity with local kombucha on green tea and AAB with *Zygosaccharomyces* sp. culture on black tea. The antioxidant capacity of kombucha was analyzed against tertiary butyl hydroperoxide-induced cytotoxicity using hepatocytes of murine and kombucha tea was found to regulate the oxidative stress-induced apoptosis in hepatocytes presumably depending on its antioxidant ability and functioning via mitochondria-dependent pathways and could be useful against oxidative stress-related liver diseases. The antioxidant capacity of kombucha tea is based on the existence of polyphenols of tea, DSL, and ascorbic acid. It has antioxidant activity higher than unfermented tea, due to alterations of tea polyphenols by enzymes synthesized during fermentation and the production of some metabolites having low molecular weight (Bhattacharya et al., 2011). The extent of this activity depends on the microbiota, substrate composition, and fermentation period, which in turn determined the feature of their metabolites. Free radical scavenging activity of this beverage demonstrated that temporal profiles. However, extended fermentation is not advised because of cumulation of organic acids, which may reach hazardous levels (Jayabalan et al., 2014). Sies and Stahl (1995) reported on vitamins E, ascorbic acid, β-carotene, and other carotenoids present in kombucha as antioxidants. Vitamin C activity as well as activities of other compounds present in kombucha fermentation system has changed in a positive way by the chemical composition of beverage. For instance, tocopherol and ascorbic acid exert intense synergetic effects on the antioxidant capacity of catechins. Therefore, vitamin C and other constituents of kombucha beverage protect human health more efficiently than the same isolated compounds (Loncar et al., 2006). Oral administration of kombucha to rats exposed to pro-oxidation species also displayed decrease in lipid oxidation and DNA fragmentation (Dufresne and Farnworth, 2000; Dipti et al., 2003).

Because of the high contents of phenolic compounds in traditional black tea, kombucha tea significantly increases the free hydroxyl and superoxide radical scavenging activities (Yang et al., 2009). Kombucha tea contains more polyphenols, having an antioxidant activity 100-fold higher than ascorbic acid and 25-fold higher than vitamin E (Adriani et al., 2011). Kombucha tea treatment potentiates the hepatic glutathione (GSH) antioxidant/detoxication system. It has been confirmed experimentally that kombucha tea decreases oxidative stress and provoked its immunosuppression (Sai Ram et al., 2010). Also vitamin C, present in kombucha tea, has a potency to support the immune system (Dufresne and Farnworth, 2000; Adriani et al., 2011). The antioxidant ability of the beverage provides protection against cell damage, development of inflammatory diseases, depression of an immunity, and origin of tumors (Vina et al., 2014).

The antioxidant capacity of kombucha have been shown against oxidative stress-induced hepatotoxicity and nephrotoxicity, as well as in diabetic rats and gastric ulceration in mice (Gharib, 2009; Banerjee et al., 2010; Bhattacharya et al., 2013; Marzban et al., 2015).

Fu et al. (2014) determined the scavenging abilities against 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical and superoxide anions as well as the total reducing power of kombucha beverages produced from low-cost green tea, black tea, and tea powder. The scavenging abilities of hydroxyl radicals and DPPH were in descending order: low-cost green tea kombucha > tea powder kombucha > black tea kombucha. Preparation of kombucha having high antioxidant capacity by a quick fermentation of low-cost green tea could be ideal and economical.

Sweetened *Melissa officinalis* L. (lemon balm) infusion could be used for the preparation of kombucha having significant antioxidant ability. When compared with kombucha made from black tea, lemon balm-based kombucha demonstrated a higher antioxidant activity against DPPH. Moreover, the antioxidant activity of lemon balm kombucha having optimum titratable acidity (0.44%) (w/v) was higher than that of lemon balm infusion. Rosmarinic acid, phenolic compounds, and metabolites produced by bacteria and yeasts are probably active components responsible for the antioxidant activity (Velicanski et al., 2014).

10.7 Energizing Ability of Kombucha Tea

Energizing ability of kombucha tea can be explained by the formation of iron (set free from tea) and gluconic acid synthesized chelating complex, which increases the level of blood hemoglobin, supply of oxygen to the tissues of the consumer, and stimulates adenosine triphosphate (ATP) synthesis. Weak organic acids and vitamin C, synthesized by kombucha, can enhance an effect on nonheme (plant-derived) iron absorption, and potency to increase the vitality; vitamin B group can promote energy regeneration by enzymatic activation of lipid and protein metabolism (Adriani et al., 2011; Vina et al., 2013).

10.8 Anticancer Properties of Kombucha Tea

The Russian Academy of Sciences and The Central Oncological Research Unit in Russia have conducted population investigations on kombucha and have determined that consumption of this beverage on a daily basis has a correlation with a strong resistance to cancer (Dufresne and Farnworth, 2000). Scientists have introduced many possible mechanisms for the anticancer property of kombucha. It was assumed that kombucha modulates immunity and prevents cancer proliferation in particular, at early phase of hormone-dependent tumor cell growth, due to the synergetic action of kombucha symbiosis products such as glucuronic acid, lactic and acetic acid, and antibiotic compounds (Danielian, 2005). It has also been declared that the capability of this beverage to act as an anticancer agent is related with the presence of tea polyphenols and the secondary metabolites produced in the course of the fermentation (Jayabalan et al., 2011, 2014). Several researches reported the ability of the tea polyphenols for inhibiting gene mutations, preventing the proliferation of cancer cells, and inducing cancer cell apoptosis and the ability for terminating metastasis that have been highlighted as possible mechanisms for the anticancer properties (Bode and Dong, 2003). It has also been noted that the ingestion of kombucha tea could help cancer patients to re-equilibrate blood pH which usually rises in the course of the illness. Moreover, cancer patients lack L-lactic acid in their connective tissues; this could also be relieved by the consumption of kombucha, where lactic acid is produced as a by-product in the course of fermentation (Dufresne and Farnworth, 2000). Many of the compounds that were found in kombucha tea such as phenolic compounds, gluconic acid, glucuronic acid, lactic acid, and ascorbic acid are known to have the ability to reduce the occurrence of gastric carcinoma. The DSL in kombucha inhibits the glucuronidase activity (an enzyme which is thought to be indirectly related to cancer) (Deghrigue et al., 2013). Glucuronidase is able to hydrolyze glucuronide in the lumen of the gut and produce cancer-causing aglycones (Kumar et al., 2015). The DSL could prevent glucosidase activity, thereby catalyze glucuronic acid to remove toxic substances (Roussin, 1996), including carcinogens [polycyclic aromatic hydrocarbons (PAHs), some nitrosamines, aromatic amines, mycotoxins), particular tumor promoters (steroid hormones) and hepatotoxins (acetaminophen)] (Dutton, 1980). Moreover, they could regulate steady-state levels of estradiol and other steroid hormones and their secretion in the form of glucuronides (Walaszer, 1993). The DSL and glucuronic acid are parts of the glucuronate pathway, and the presence of DSL could reflect the presence of glucuronic acid (Yang et al., 2010). It has been found out that the polyphenols of kombucha have antitumor properties, thus acting as a cancer-blocking agent (Deghrigue et al., 2013). Another study reported that dimethyl 2-(2-hydroxy-2-methoxypropylidene) malonate and vitexin containing ethyl acetate fraction of kombucha caused cytotoxic effects on human renal carcinoma and human osteosarcoma (Jayabalan et al., 2011). The influence of kombucha tea on the development of angiogenesis-dependent prostate cancer demonstrates that kombucha tea significantly reduces prostate cancer cell development and metastasis by downregulating the expression of angiogenesis stimulators. This data propose that kombucha may be helpful for the inhibition of prostatic adenocarcinoma and treatment (Srihari et al., 2013b). The effects of kombucha on viability and invasiveness of various cancer cell lines, such as human lung carcinoma, human osteosarcoma, and human renal cell carcinoma has been studied. It has been confirmed that matrix metalloproteinases (MMPs) play a part in these processes and is linked to tumor metastasizing. Kombucha tea inhibits MMPs in a concentration-dependent manner. Malonate and vitexin have been purified from an ethyl acetate fraction of kombucha tea and the researchers have proven experimentally that these two compounds may be responsible for cytotoxic and antiinvasive effects of kombucha (Jayabalan et al., 2011). It was mentioned that folic acid present in kombucha tea modulates cellular cycle and cell proliferation, and can play a role in cancer development (Figueiredo et al., 2009).

10.9 Kombucha Tea as Hepatoprotective Agent

Detoxifying property of kombucha is probably related to the capability of glucuronic acid to bind to toxic compounds and to increase their excretion from the kidneys or the intestines (Afsharmanesh and Sadaghi, 2014). Glucuronic acid is produced due to the oxidation of glucose in due course of fermentation (Vijayaraghavan et al., 2000). Glucuronic acid is well established as an important detoxicant in the protection of health; thus, it is able to bind to the toxins in the liver and induce them to flush out of the body (Vina et al., 2013; Nguyen et al., 2014). Malic acid also helps in detoxifying the liver (Srihari and Satyanarayana, 2012). Moreover, kombucha tea consumption is also known to aid excretion of heavy metals and environmental contaminants from the human body through the kidneys (Teoh et al., 2004). It is also effective in the biotransformation of indigenous metabolites (i.e., excess of steroid hormones and bilirubin) (Srihari and Satyanarayana, 2012). Kombucha reduces hepatic detoxication to less than half of the normal capacity and inhibits the paracetamol-induced hepatotoxicity (Pauline et al., 2001). Jalil et al. (2012) indicated that kombucha prevents acetaminophen-induced hepatotoxicity in mice and might provide a beneficial therapy for intoxicated patients. Kombucha tea is anticipated to ameliorate liver damage induced by aflatoxin. The mechanism of hepatoprotection, observed during kombucha fermented black tea treatment, might be due to high antioxidant ability of black tea and the presence of glucuronic acid (Jayabalan et al., 2010). Kombucha tea hepatoprotective and curative properties against toxicity, induced by carbon tetrachloride (CCl_4) (xenobiotic that induces lipid peroxidation and causes liver cell damage), have been studied (Murugesan et al., 2009). CCl_4 significantly increases the levels of liver marker enzymes ALAT, ASAT, ALP, and MDA. Kombucha tea tends to decrease them; treatment period upgrowth intensified decrease in marker enzyme levels. The regeneration/reverting rate of liver necrotic tissues to normal condition has been more pronounced in black tea kombucha fed animals when compared to animals fed by unfermented black tea. The scientists have assumed that kombucha tea's antioxidant properties can be the main cause for the influential hepatoprotective and remedial properties against CCl_4-induced hepatotoxicity (Kumaravelu et al., 1995). Hepatoprotective efficiency of kombucha against paracetamol is majorly originated from the existence of DSL (Wang et al., 2014). Results of the research by Hyun et al. (2016) demonstrated that kombucha tea protected the liver from damage, decreased lipid accumulation, and promoted liver amelioration in mice. Their findings suggest kombucha beverage as a prospective nutritional strategy for inhibiting the progression of nonalcoholic fatty liver disease.

10.10 Antimicrobial Properties of Kombucha Tea

Kombucha tea was investigated for its inhibitory activity on some pathogens. It could inhibit the growth of *Enterobacter cloacae, Aeromonas hydrophila, Agrobacterium tumefaciens, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Salmonella typhimurium, Salmonella enteritidis, Shigella sonnei, Staphylococcus epidermidis, Staphylococcus aureus, Yersinia. enterocolitica, Campylobacter jejuni, Helicobacter pylori*, and *Candida albicans* (Sreeramulu et al., 2000, 2001; Battikh et al., 2012). Acetic acid has been proposed to be the principal antimicrobial agent (Yang et al., 2009), bacteriocins and tea-derived phenolics may also be involved (Sreeramulu et al., 2001). Tietze (1995) reported the presence of usnic acid (an antibacterial compound) in kombucha cultures. Battikh et al. (2013) determined that black and green tea kombucha had antimicrobial activity against the tested pathogens, and the kombucha made by green tea had the maximum antimicrobial potential. Kombucha has antifungal activity against a wide range of pathogenic *Candida*. Studies on kombucha have reported its antimicrobial potential against both Gram(+) and Gram(−) pathogens. Antimicrobial activity of the beverage especially results from acetic acid, proteins, and catechins. Sreeramulu et al. (2000) reported that a number of Gram(+) and Gram(−) bacteria was inhibited by acetic acid and catechins. Kombucha prepared from lemon balm (*Melissa officinalis* L.) exerted significant antimicrobial activity against Gram(+) and Gram(−) bacteria. However, the growth of yeasts and molds was not inhibited (Cetojevic-Simin et al., 2012). Also, Velicanski et al. (2014) indicated that kombucha prepared from lemon balm exerted antibacterial activity against pathogenic Gram(+)

(*Staphylococcus saprophyticus, Staphylococcus equorum, Bacillus* sp., *Listeria. monocytogenes* and *Listeria innocua*) and Gram(−) bacteria (*P. aeruginosa, Proteus* sp., *E. coli, Citrobacter freundii, Enterobacter cloacae* and *Salmonella* sp.).

Concentration of acetic acid in kombucha affects directly its antimicrobial activity. At the same time, the antibacterial activity of neutralized kombucha against several bacteria indicates that some nonacidic compounds of kombucha may contribute to antimicrobial activity (Velicanski et al., 2014). Researches on the antimicrobial activity of kombucha indicated the presence of antimicrobial agents other than organic acids or enzymes produced as well as tannins preliminarily present in the tea (Sreeramulu et al., 2001). It has also been noted that kombucha tea contains strong water-insoluble bacteriocins with activity against broad spectrum of infectious diseases, such as influenza, scarlatina, typhoid, paratyphoid fever, diphtheria, and dysentery (Danielian, 2005). Ismaiel et al. (2016) demonstrated that kombucha inhibits patulin formation by *Penicillium expansum, Talaromyces purpureogenus*, and *Acremonium implicatum* in a liquid medium and in apple fruit. The inhibitory activity was dose-dependent. Moreover, kombucha bio-cellulosic layer could be utilized as a biosorbent for the removal of patulin from aqueous medium. For this reason, kombucha seems to be up-and-coming safe alternative bio-preservative offering a protection against patulin intoxication.

Battikh et al. (2012) investigated and compared the antimicrobial activity of kombucha prepared from black tea with kombucha analogues produced from extracts of *Thymus vulgaris L., Lippia citriodora, Rosmarinus officinalis, Foeniculum vulgare,* and *Mentha piperita*. Strong antimicrobial potentials were found with kombucha prepared by the fermentation of *L. citriodora* and *F. vulgare* infusions, exhibiting the most significant inhibition zone observed against *Candida glabrata, C. tropicalis, Candida sake, Candida dubliniensis,* and *C. albicans*.

Ayed and Hamdi (2015) tested antimicrobial activity of kombucha made by cactus pear juice against *S. aureus, B. cereus, S. epidermidis*, and *Enterococcus faecalis, E. coli, Klebsiella pneumoniae*, and *P. aeruginosa*. These beverages had antimicrobial activity against all pathogens tested. Contrarily, no test microorganisms showed vulnerability to the unfermented juice component. The antimicrobial activity was due to the organic acids, primarily acetic acid, and was eliminated when samples were neutralized.

Mahmoidi et al. (2016) investigated the antifungal activity of ethyl acetate fraction of kombucha tea (KEAF) against *Malassezia* species obtained from the patients suffered from seborrheic dermatitis. Because KEAF demonstrated inhibitory activity against *Malassezia* species, it can be promoted as supplementary medicine for the treatment of the *Malassezia* infections.

10.11 Probiotic Effects of Kombucha Tea

Many studies have claimed that kombucha tea acts as a symbiotic, a combination of prebiotics and probiotics (Dufresne and Farnworth, 2000; Junior et al., 2009). The bacteria and yeast present in this beverage act as probiotics and the supernatant bio-cellulose network can help the growth of the beneficial microorganisms present in the intestine. The popularity of this beverage has increased since scientists have reported that kombucha could be used to give the required nutrition and help sustain health and wellness in humans who work under unhealthy and challenging environments, such as workers in mines and polar expeditors. When the human body is exposed to such conditions for prolonged periods, the normal microbial consortium in the intestine changes due to the labored conditions, psychoemotional disturbance, and forcible change in the diet. This may lead to the disappearance of the protective gut microorganisms and the immergence of harmful secondary infections by opportunistic microorganisms. This shift in the gut microbiota could lead to many health problems such as allergies, autoimmune diseases, multiple sclerosis (MS), and transplant infectious disease. The change in the gut microbiota could be meliorated partially with the help of kombucha. In light of the above-mentioned possibilities, researchers have started to take into account this beverage to be consumed by astronauts as a dietary supplement (Kozyrovska et al., 2012).

10.12 Kombucha Tea as Antihypercholesterolemic Agent

Kombucha tea as an antihypercholesterolemic agent has been extensively studied on animals and in some clinical trials with humans. Particular mechanisms of kombucha tea antihypercholesterolemic action have been argued, antioxidants are known to be a major factor for antihyperlipidemia. Suppression of oxidative stress has been shown as a way of reduction of hypercholesterolemic atherosclerosis (Dufresne and Farnworth, 2000; Yang et al., 2009). Kombucha tea contains more polyphenols than its unfermented tea substrate, as well vitamins E and ascorbic acid, is well known as high-level antioxidants (Yang et al., 2009; Kallel et al., 2012). Therefore, traditional kombucha tea has been proven experimentally in vivo to protect from atherosclerotic disorders, contributing to lipoprotein-bound antioxidant activity. It has been mentioned that kombucha tea reduces atherosclerosis through the regeneration of cellular walls (Dufresne and Farnworth, 2000). The relationship between coronary heart disease and lowering of cholesterol has been mentioned (Dufresne and Farnworth, 2000; Yang et al., 2009).

Kombucha tea that has a high level of glucuronic acid has been used to evaluate a decline in blood cholesterol in rats. The results obtained establish the ability of kombucha tea to decrease blood plasma total cholesterol as much as 45%–52%; triglyceride values declined to 18%–27%, LDL-Ch 75%–91%, with HDL-Ch values increasing to 18%–27% (Suhartatik et al., 2011). Kombucha tea could reduce blood cholesterol levels through the inhibition of HMG (3hydroxy 3-metilglutaril CoA reductase) which is a cholesterol synthesis enzyme and/or through the mechanism of increased excretion of cholesterol. Kombucha tea induced a significant retardation in the absorption of LDL-Ch and triglycerides and a marked increase in HDL-Ch in rats (Aloulou et al., 2012). The studies on cats and dogs have shown the strengthened myocardial contractions after using kombucha tea becoming more frequent and with amplitude increases (Danielian, 2005).

Bellassoued et al. (2015) compared free-radical scavenging abilities and polyphenol contents of green tea and kombucha tea. Wistar rats fed cholesterol-rich diets were treated by kombucha tea or green tea (5 mL/kg body weight per day, po) for 4 months. Phenolic compounds of kombucha tea was 955 ± 0.75 mg GAE/g followed by green tea (788.92 ± 0.02 mg GAE/g). The free radical scavenging activity of kombucha tea was greater than green tea. Compared to green tea, kombucha induced reduced serum levels of total cholesterol, triglycerides, very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) by 26%, 27%, 28%, and 36%, respectively, and increased the serum level of high-density lipoprotein cholesterol (HDL-C). Kombucha tea induced a 55% decrease in thiobarbituric acid reactive substances level in liver and 44% in kidney, compared with those of animals fed a cholesterol-rich diet alone. Besides, catalase and superoxide dismutase activities were reduced by 29% and 33%, respectively, in liver and 31% and 35%, respectively, in kidney, after oral administration of kombucha tea, compared with those of cholesterol-rich diet fed rats. Their results indicated that kombucha tea administration induced attractive curative effects on hypercholesterolemic rats, particularly in terms of liver-kidney functions.

10.13 The Effects of Kombucha Tea on Nervous System

Several amino acids and methylxanthine alkaloids (i.e., caffeine, theophylline, and theobromine), ascorbic acid and the B group vitamins including folic acid, necessary for normal metabolism in the nervous system are determined in kombucha tea (Dufresne and Farnworth, 2000; Adriani et al., 2011; Kallel et al., 2012; Vina et al., 2014).

Kombucha tea has been offered as a prophylactic functional beverage against headaches and dizziness caused by hypertension, and kombucha tea consumption is recommended for hypertension treatment (Danielian, 2005). Nervous system pathologies have drawn attention to specific glucuronic acid containing glycans in the context of the nervous system, providing insights into their possible functional roles (Kleene and Schachner, 2004).

Kombucha can help with headaches, irritability, insomnia, and epilepsy prevention. It has been advised that kombucha tea intake may improve temper, and prevent depression in the elderly (Danielian, 2005). Marzban et al. (2015) tested the therapeutic effectiveness of kombucha in experimental model of MS. Their results showed that kombucha had beneficial effects such as lower incidence of experimental autoimmune encephalomyelitis, modulation in the severity, and also retardation in the disease initiation. The inflammatory criteria in kombucha tea treated mice were significantly lower than the control group. Moreover, the serum levels of nitric oxide and tumor necrosis factor-alpha in kombucha tea dosed mice have significantly reduced when compared to control group. Kombucha with its potential therapeutic effects and immunomodulatory characteristics might be proposed, after additional necessary experiments and clinical trials, for the treatment of MS (Suhartatik et al., 2011).

10.14 Use of Different Substrates in Kombucha Fermentation

It has been demonstrated that green tea has better stimulation potency on the fermentation compared with black tea. For this reason, beverage formation occurs in a shorter period (Velicanski et al., 2007). This beverage has been prepared rarely by jasmine tea, lemon balm tea, peppermint tea, and mulberry tea (Velicanski et al., 2007, 2013; Jayabalan et al., 2014). Although Hoffmann (2000) declared that herbal teas were not very suitable for making kombucha due to the deficiency of purine derivatives. Cvetkovic (2008) demonstrated that echinacea tea or winter savory tea has more stimulative effects on the fermentation than black tea, yielding a product in a shorter period. Moreover, as a nitrogen source for kombucha fermentation lemon balm tea can be successfully used (Cetojevic-Simin et al., 2012). All of these herbs contain many bioactive compounds, provide the desired antioxidant ability, and prevent diseases related with oxidative stress (Cetkovic et al., 2007; Velicanski et al., 2014). These functional herbs can be successfully used in combination with black or green tea for kombucha fermentation, yielding a beverage of strong antioxidant activity and antimicrobial property (Essawet et al., 2015).

Battikh et al. (2012) used *Thymus vulgaris* L., *L. citriodora, R. officinalis, F. vulgare,* and *Mentha piperita* as substrates for kombucha production. Authors determined that different herbs can be evaluated as substrates for fermentation (Velicanski et al., 2013). The fermentation of sweetened *Q. resinosa* infusions was investigated by Vazquez-Cabral et al. (2014). The metabolic consumption of flavan-3-ols and hydroxybenzoic acid analogues and the production of succinic acid along with the decreased fermentation period and higher sugar content improved the acceptability of the beverages. Malbasa (2004) reported some trials on the use of nontraditional ingredients such as Coca-Cola, wine, vinegar, Jerusalem artichoke, *Echinacea*, *Mentha*, milk, fresh and reconstituted sweet whey, acid whey and more for the kombucha production. It was determined that kombucha beverage prepared from the extract of Jerusalem artichoke tuber could be suitable due to the low sugar contents, and the presence of dietary fibers such as inulo-oligosaccharides (Malbasa et al., 2002a; Loncar et al., 2007). Actually, fermentative substrates contain similar metabolites as the beverage with saccharose, also supplements like prebiotics (i.e., fructo-oligosaccharides and inulin), to support the quality of the beverage (Malbasa et al., 2002a). Talawat et al. (2006) cultivated kombucha on Japanese green tea, oolong tea, jasmine tea, and mulberry tea. Yavari et al. (2011) examined the fermentation of sweetened sour cherry kombucha. Sugar beet molasses is attractive substrate for kombucha fermentation depending on price advantage and the availability of minerals, organic compounds, and vitamins (Loncar et al., 2001; Malbasa et al., 2002a,b; Rodrigues et al., 2006). Use of lactose as carbon source for the kombucha fermentation is proved (Reiss, 1994). Belloso-Morales and Hernandez-Sanchez (2003) used whey for kombucha production. It is possible to obtain a fermented dairy product when kombucha is used as a novel type of starter culture in milk fermentation (Vitas et al., 2013; Malbasa et al., 2015). Malbasa et al. (2009) determined that kombucha could be produced from cow milk. Vitas et al. (2013) also reported that by using kombucha obtained by cultivation on extracts of sweetened winter savory (*Satureja montana*) and stinging nettle (*Urtica dioica*), fermented milk beverages could be prepared. Malbasa et al. (2014) determined the antioxidant capacity of fermented dairy products obtained by using kombucha produced by fermentation on sweetened wild thyme (WT) extract. Milk containing 0.8, 1.6 and 2.8% milk fat was used and 37°C, 40°C, and 43°C were tested as fermentation temperature. When the pH reached 4.5, fermentation was terminated. The WT containing products demonstrated opposite antioxidant response to DPPH and hydroxyl radicals in terms of milk fat. In terms of antioxidant capacity, optimum processing conditions for WT products were 2.78% milk fat and fermentation at 37°C. However, these parameters are completely different

(1.10% milk fat and fermentation at 43°C) in order to obtain WT products with a high sensory score.

Hrnjez et al. (2014) evaluated the effects of using kombucha as starter culture for milk fermentation. The milk product fermented by kombucha indicated similar trend of changes in pH, degree of proteolysis, and organoleptic properties as the products obtained by probiotic and yoghurt starters. Significant angiotensin-converting enzyme (ACE) inhibitory activity was determined in all the fermented products, which increased during storage. Fermented dairy product obtained by kombucha inoculum demonstrated the highest ACE activity (63.43%) at the end of 14 days compared with probiotic and yoghurt products. Higher 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) than DPPH radical scavenging activity was measured in all the samples, while both activities reduced slightly during storage.

Sun et al. (2015) suggested that intake of kombucha fortified with wheatgrass juice is worthwhile over conventional kombucha recipes in terms of supplying various phenolic compounds and might have more potential to reduce oxidative stress.

Watawana et al. (2015b) investigated if the antioxidant ability of coffee could be enhanced by inoculation of the kombucha. The amount of chlorogenic acid and caffeic acid showed statistically significant increases ($P < 0.05$). Also, α-amylase inhibitory activity increased by fermentation. As a result, this research provided preliminary evidence on the improvement of the antioxidant and starch hydrolase inhibitory potential of coffee beverages by kombucha fermentation.

Essawet et al. (2015) investigated the contribution of CoffeeBerry extract on kombucha fermentation and antioxidant capacity of beverage. CoffeeBerry extract contributed to a faster fermentation of cultivation medium. All samples enriched with CoffeeBerry had chlorogenic acid and demonstrated remarkable antioxidant activities on DPPH and toxic OH radicals.

Watawana et al. (2016) determined improvement in antioxidant capacity and starch hydrolase inhibitory activity of king coconut water fermented with kombucha. The increase in total phenolics and the antioxidant activity was statistically significant ($P < 0.05$) from day 1 onwards in the fermented beverage. Also, the starch hydrolase inhibitory activity had statistically significantly raised ($P < 0.05$) by day 7.

Ayed and Hamdi (2015) produced kombucha analogue from cactus pear juice to formulate a novel beverage with improved nutritional potential. pH is reduced from 5.1 to 3.5 and 23% increase in total phenolic compound was observed by the growth of microflora. The antioxidant capacity of the sample was also enhanced, reaching 81% and 65% as determined, respectively, by DPPH and ABTS assays. The increased antioxidant activity was attributed to betalains in cactus pear and to certain metabolites formed during fermentation.

Ebrahimi Pure and Ebrahimi Pure (2016) produced kombucha beverages using nettles leaf and banana peel infusions. Kombucha analogue from banana peel had the highest antioxidant activity by inhibiting 94.62% of DPPH. They reported that banana peel, as a waste herbal material, and nettles leaf are good ingredients for being used as substrate to prepare kombucha beverage.

Watawana et al. (2017) used different sweeteners for kombucha production. The phenolics and antioxidant capacity and starch hydrolase inhibitory activity were investigated. Brown sugar, glucose, and sucrose were the most effective sweeteners in terms of high antioxidant and starch hydrolase inhibitory activities. However, fermentation was not inhibited by the use of bees' honey, *Caryota urens* honey and palmyrah jiggery (*Borassus flabellifer*).

Vazquez-Cabral et al. (2017) studied the antioxidant activity and antiinflammatory effects of kombucha analogues from oak. The beverage had good antioxidant properties attributed particularly to their phenolic profile. The major effect was detected in their ability to suppress lipopolysaccharide-induced production of nitric oxide, TNF-alpha and IL-6, showing an important in antiinflammatory activity.

10.15 Toxicological Concern of Kombucha Tea

Even though kombucha beverage has therapeutic effects, there is limited evidence on toxicity concern that it may have serious health risks, especially in the immune-deficient peoples (Kole et al., 2009; Jayabalan et al., 2014). Some people reported dizziness and nausea following kombucha consumption. Also, two cases of unexplained serious illness reported after kombucha consumption. Kombucha tea is contraindicated in pregnant and breastfeeding mothers. Gastrointestinal toxicity and plumbism was determined in two persons (Anonymous, 1995). Sadjadi (1998) isolated *Bacillus anthracis* in unhygienically fermented kombucha. Furthermore, Gamundi and Valdivia (1995) reported the risks of consuming of kombucha tea by HIV-positive patients. Although a majority of people drinking kombucha tolerate it well, and without symptomatic adverse effects, HIV patient's experience of kombucha consumption indicates the potentially hazardous consequences (Kole et al., 2009). Also, allergic reactions, jaundice, nausea, vomiting, neck and head pain related to kombucha consumption were reported in four patients (Srinivasan et al., 1997). Symptomatic lead poisoning was reported in a couple who had been consuming kombucha fermented in a ceramic jug for six months (Phan et al., 1998). It was approved that organic acids in the beverage resolved lead from the glaze used in the ceramic teapot. Sabouraud et al. (2009)

declared the cases of plumbism depending on the use of lead glazed ceramic pot for storing kombucha. Use of glass containers is advised for the production and storage of kombucha to prevent leaching of toxic compounds into the beverage (Jayabalan et al., 2007, 2014). One case of possible hepatotoxicity (Perron et al., 1995) and one case of skin disease (Sadjadi, 1998) were also reported. "47-year old female patient was hospitalized and has consumed daily ounces of kombucha tea. During hospitalization patient was diagnosed with toxic hepatitis and treated with intravenous solutions of hepatic protective and ursodeoxycholic-acid (effective therapy)" (Kovacevic et al., 2014). Medical examinations indicated that kombucha tea has potential to revert hepatic toxicity induced by CCl_4. However, it could induce toxicity when it is consumed in overdose. People suffering from severe affliction should be conscious of side effects caused by kombucha consumption (Dufresne and Farnworth, 2000). Microbiota in the bio-cellulose pellicle of kombucha are able to form a strong symbiosis which can prevent the growth of contaminating bacteria (Dufresne and Farnworth, 2000; Khan and Mukhtar, 2013). However, pathogens could contaminate the kombucha tea in the course of the preparation. pH of the beverage reaches ≤ 4.2 due to fermentation. Nevertheless, there is a high possibility for contaminations to occur until this is achieved. *Penicillium* and *Aspergillus* contamination could occur, when the kombucha tea is homemade (Kole et al., 2009). Therefore, it is very important to be precautious when administering infected beverage by immunocompromised individuals. Several researches have substantiated that kombucha could cause nausea, difficulty in breathing, headache, dizziness, and jaundice (Vijayaraghavan et al., 2000; Kole et al., 2009). Further researches should be done to form a substantial conclusion about the safety aspects in the consumption of kombucha tea. In order to prevent any contamination, clean and sanitized utensils and vessels should be used during the preparation of kombucha tea. A clean and sanitized porous cloth should be used for covering of fermentation vessel. Preparation and fermentation areas have to be kept clean and sanitary to control microbial growth since tea must be cooled down to about 20°C within 2 h prior to inoculation of the kombucha culture. Because overproduction of acetic acid could be hazardous, monitoring and control of pH level during the fermentation process is very important. According to the British Columbia Center for Disease Control, the fermentation is targeted to finish below pH 4.2. Overproduction of acetic acid is controlled by stopping the further development of acetic acid when the pH approaches 2.5. This is fulfilled by refrigerating the fermented kombucha tea and keeping the beverage refrigerated until consumed (Anonymous, 2015).

The Food and Drug Administration (FDA) states that when safely prepared, kombucha can be safe for human consumption.

Vijayaraghavan et al. (2000) investigated the subacute oral toxicity potency of kombucha by monitoring body weight, feed-water consumption, general behavior, and histological examinations of rats. Researchers reported that kombucha administration to rats for 3 months did not display any toxic symptoms. Hematological and biochemical data of experimental animals investigated were within clinical limits. Kombucha tea toxicity was investigated by feeding the rats with three different doses (normal dose and 5 and 10 times that dose) of kombucha for 15 days and by testing several parameters (Pauline et al., 2001). It was determined that kombucha demonstrated no significant toxicity. Authorities should focus their attention on the establishing global policies and regulations regarding the safety of kombucha consumption and serial production (Watawana et al., 2015a).

10.16 Conclusion

Traditional kombucha tea has been consumed for its supposed health-promoting benefits and energizing features. The relationship between kombucha consumption and improved health and the responsible mechanisms are being evaluated to shed light on the claimed health benefits. Because medical researches of kombucha tea's role in human health and wellness are limited, risks have to be taken into account. However, FDA states that when safely produced, kombucha can be safe for human consumption.

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MILLET BASED FERMENTED BEVERAGES PROCESSING

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11.1 Summary

Cereals particularly millet has the greatest potential of being used as an industrial raw material. However, research efforts on its utilization as food for human consumption are still at their infancy. Many different beverages from millets have been made by fermentation using microorganisms such as yeasts, lactic acid bacteria, and acetic acid bacteria. The final products should be made acceptable and affordable to the general consumers. In many cases, malt, germinated millet, is the main source of these beverages to enhance the amylolytic enzymes for starch degradation. Usually, lactic acid bacteria and yeast are involved in spontaneous fermentation. This chapter focuses on the function assumed by fermentations in most cases of millet-based fermented beverages, the product name, class, nutritional value, and responsible fermenters of these cereal beverages. Furthermore, the sociocultural and localization and health-enhancing aspects of these beverages were also reviewed. Indeed, indigenous millet brewing technology is similar to the modern brewing technology except for the differences in the scale of the level of the technology used. Product obtained from millet-based fermented beverages include *Bensaalga, Boza, Burukutu, Bushera, Doro, Fura, Jandh, Kodo ko jaanr, Koko, Koozh, Kunun-zaki, Madua Apong, Malwa/Ajon, Mangisi, Ogi, Oshikundu, Oti-oka, Shakparo Ale, Togwa, Uji, Zoom-koom, Xiao mi jiao*, etc.

11.2 Introduction

Worldwide, cereals are the most important source of food and play a great role in constituting the human diet. In particular, they are an important source of starch and other dietary carbohydrates (dietary

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fiber), nutrients required for generating energy. Besides wheat, rice, and maize, millet is the major food source for millions of people, especially those living in hot, dry areas. Millet is mostly grown in areas with marginal agricultural conditions under which major cereals do not give substantial yields (Amadou et al., 2013a; Dayakar Rao et al., 2017). Along with maize, sorghum, and Coix (Job's tears), millet is classified under the grass subfamily Panicoideae (Yang et al., 2012). Millet is a word derived from the word mille, which signifies a thousand grains. The most widely grown millet species in the world is pearl millet (*Pennisetum glaucum* L. R. Br.), followed by foxtail (*Setaria italica* L.), proso (*Panicum miliaceum*), and finger millet (*Eleusine coracana*) (Chandra et al., 2016; Shahidi and Chandrasekara, 2013). Millet count is enough as food for many underdeveloped countries considering that it can grow under adverse weather conditions like limited rainfall. In contrast to other cereals, millet is composed of high fiber content, protein quality, and mineral composition that significantly contribute to nutritional security of a large section of population living in the millet growing areas, the most disadvantaged groups (Taylor, 2017b). Furthermore, not only is millet highly nutritional but also superior to the major cereals in terms of protein, energy, vitamins, and minerals (Capozzi et al., 2012; USDA, 2017). In addition, millet is suggested as a good source of dietary fiber, phytochemicals, micronutrients, and nutraceuticals (Table 11.1) Millet grains are round shaped seeds naturally available in various colors and sizes, depending on the variety.

Table 11.1 Composition of *Kodo* and *Ragi* Millets (per 100 g Edible Portion, 12% Moisture Content)

S/N	Particulars	*Kodo* Millet	*Ragi* Millet	Wheat	Rice
1.	Carbohydrate (g)	66.6	72.6	71.2	78.2
2.	Protein (g)	9.8	7.7	11.8	6.8
3.	Fat (g)	1.3	1.5	1.5	0.5
4.	Crude fibre (g)	9.0	3.6	12.9	5.2
5.	Minerals (g)	2.6	2.7	1.5	0.6
6.	Calcium (mg)	27	344	41	10
7.	Phosphorus (mg)	188	250	306	160
8.	Iron (mg)	5.0	6.3	3.9	0.5
9.	Manganese (mg)	3.3	3.5	13.3	1.0
10.	Magnesium (mg)	228	130	120	32

Source: USDA (2017) Nutrient database. Available from: http://en.wikipedia.org/wiki.

There is no doubt that millet is considered as an ancient food, for instance, the widespread cultivation of proso millet in Kazakhstan dates back more than 7000 years ago (Miller et al., 2016; Yang et al., 2012) whereas cultivation of pearl millet in Ghana dates back more than 5000 years ago (Spengler et al., 2014). It is probable that pearl millet, fonio, finger millet, and teff all originated in Africa, south of the Sahara; pearl millet and fonio in West Africa and finger millet and teff in Northeast Africa. Finger millet and pearl millet were brought to the Indian subcontinent around 2000 BP (Adekunle, 2012). All the other millet species probably originated in Eurasia. However, evidence suggests that proso millet existed across Asia and Europe prior to 7000 BP and presence of foxtail millet has also been reported in Northern China (Taylor, 2017b; Yang et al., 2012). Currently, millet is the sixth most important cereal grain in terms of production after maize, rice, wheat, barley, and sorghum. According to FAO reports (FAO/FAOSTAT, 2014), the total yield of millet is estimated to be 28.385 million tonnes, with Asia and Africa considered as the highest contributors, producing almost 95% of the global yield. India was reported to be the highest producer of millet in Asia producing 11.420 million tonnes followed by Niger as the top producer in Africa producing 3.321 million tonnes, followed by China and Mali (Fig. 11.1).

The rapid increasing world human population emphasizes the urgent need for increased food production thus worthy of exploring plants such as millet that are grown locally and consumed by

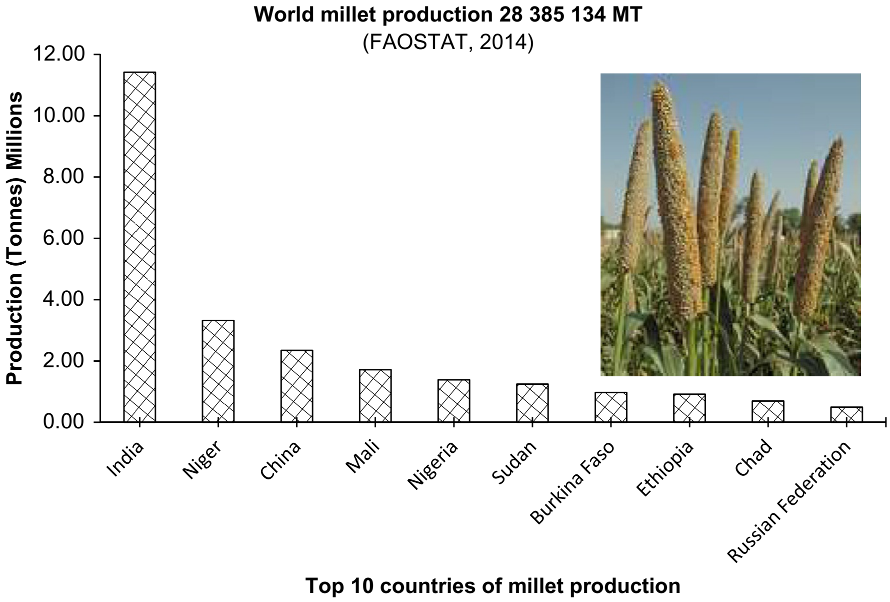

Fig. 11.1 Leading millet-producing countries in the world (FAO/FAOSTAT, 2014).

low income households in places like India and sub-Saharan Africa (Arendt and Zannini, 2013). With regard to health-enhancing properties, millet is gluten-free cereal. However, the Codex Alimentarius Commission set gluten-free standards foods for international trade purposes to the standard that gluten free foods cannot contain wheat, rye, barley, oats or their crossbred varieties, unless they have been specially processed to reduce the gluten level to below 20 ppm (Codex Alimentarius Commission, 2009; Hager et al., 2014). Published research have shown that fermented millet-based products from countries of millet production are also found in other countries with different names. This means that the processes are replicated in other countries under different name. In general cereals based fermented beverages are worldwide known in particular millet-based beverages are widely consumed in the form of traditional staple foods and used to be traditionally produced millet-based beverages industrialization remains insufficient when compare to others cereals based products (Amadou et al., 2011; Dalton, 2017). Despite being the sixth in the world cereals and the most consumed cereal food in the underdeveloped part of this world, research on millet-based fermented beverages and its potential in the industries remains in its infancy. Therefore this review wishes to contribute to the general audience on the available millet-based fermented beverages and process. This further demonstrates what researchers have done and what their recommendations (Table 11.2).

11.3 Health-Enhancing Attributes

The nutritional and health-promoting properties of millet a gluten-free and low-cost cereal have increased interest toward its consumption (Miller et al., 2016). For instance a report from Saleh et al. (2013) indicated that millet has hypoglycemic characteristics, antioxidant and antimicrobial properties attributed to its polyphenols and proteins. Millet is also recommended as an alternative for celiac patients who require gluten-free foods (Amadou et al., 2013a; Shahidi and Chandrasekara, 2013). Considering that millet does not contain gluten and has a low glycemic index (Dayakar Rao et al., 2017), studies have been undertaken to explore the potential of producing millet fermented products such as alcoholic drink, porridges, and pasta, as an alternative to gluten-rich cereal-based drinks (Serna-Saldivar, 2016). Celiac disease is known as immune reaction to eating gluten; thus, people living with this disease have to adapt their diet to gluten-free foods to enhance their living standards. Nowadays, more endogenous foods and beverages have come into focus (Amadou et al., 2011; Barmeyer et al., 2017; Christensen et al., 2017).

Table 11.2 Process Millet-Based Fermented Beverages Products

Vernacular/ Common Name	Class	Location (Region/ Country)	Strains	References
Ben-saalga	Nonalcoholic beverage	Burkina Faso	*Lactobacillus* spp and *Lactococcus* spp, *S. cerevisiae*	Muyanja et al. (2003), Tou et al. (2006), and Hassani et al. (2015)
Boza	Alcoholic beverage	South Russia, East European countries, Middle East, Northern Persia	*Lactobacillus* and *Leuconostoc genera*, *Saccharomyces*	Arici and Daglioglu (2002), Evliya (1990), and Hancioğlu and Karapinar (1997)
Burukutu	Alcoholic beverage	West Africa	*Leuconostoc mesenteroides*, *Lactobacillus*, *S. cerevisiae*, *Rhodotorula glutinis*, *C. pelliculosa*, *Cryptococcus albidus*	Blandino et al. (2003) and Jimoh et al. (2012)
Bushera	Non-alcoholic beverage	Uganda	*Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus and Streptococcus. Lactobacillus brevis*	Muyanja, et al. (2003) and Kandylis et al. (2016)
Doro	Nonalcoholic beverage	Zimbabwe	Lactic acid bacteria	Gadaga et al. (1999) and Benhura and Chingombe (1989)
Fura	Nonalcoholic beverage	West Africa	*Enterobacter*, *bacillus*, *Staphylococcus*, *Fusarium culmorum*, *A. orizae*, *A. niger*, *A. flavus A. parasiticus* and *Mucor racemosus*	Inyang and Zakari (2008), Durojaiye et al. (2010), Pedersen et al. (2012), and Jideani et al. (2010)
Jandh	Alcoholic beverage	Nepal	*Lactic acid bacteria*, *yeast* and *mold*	Freire et al. (2017), Hassani et al. (2015), and Dahal et al. (2005)
Kodo ko jaanr	Alcoholic beverage	India, Nepal and Bhutan	*Pichia anomala*, *Saccharomyces cerevisiae*, *Candida glabrata*, *Saccharomycopsis fibuligera*, *Pediococcus pentosaceus* and *Lactobacillus bifermentans*	Thapa and Tamang (2004) and Tamang et al. (1996)
Koko	Nonalcoholic beverage	West Africa	*Weisella confusa* and *Lactobacillus fermentum*, *Lactobacillus salivarius* and *Pediococcus* spp.	Lei and Jakobsen (2004) and Geetha (2013)

Continued

Table 11.2 Process Millet-Based Fermented Beverages Products—cont'd

Vernacular/ Common Name	Class	Location (Region/ Country)	Strains	References
Koozh	Alcoholic beverage	India	*Lactobacillus spp* and *Lactococcus* spp, *S. cerevisiae*	Ilango and Antony (2014) and Angmo et al. (2016)
Kunun-zaki	Nonalcoholic beverage	Nigeria, Niger and Tchad	*L. fermentum*, *L. leichmannii L. plantarum*, *L. pentosus*, *L. cellbiosus*, *Pediococcus pentosaceus* and *Leuconostoc Mesenteroides*, *Candida mycoderma* and *S. cerevisiae*	Nwachukwu et al. (2010) and Amusa and Odunbaku (2009)
Madua Apong	Alcoholic beverage	India	*Lactobacillus* spp and *Lactococcus* spp, *S. cerevisiae*	Shrivastava et al. (2015)
Malwa/Ajon	Nonalcoholic beverage	Uganda	*Lactobacillus* spp and *Lactococcus* spp, *S. cerevisiae*	Muyanja et al. (2010) and Mwesigye and Okurut (1995)
Mangisi	Nonalcoholic beverage	Zimbabwe, Uganda	Lactic acid bacteria	Zvauya et al. (1997), Benhura and Chingombe (1989), and Gadaga et al. (1999)
Ogi	Nonalcoholic beverage	Nigeria	*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus* spp., *Saccharomyces cerevisiae*, *Candida mycoderma*, *Rhodotorula* spp., *Aerobacter cloacae*, *Candida krusei*, *Debaromyces hasenu*, *Klebsiella* spp., *Staphylococcus* spp.	Abdalbasit et al. (2017), Inyang, and Idoko (2006), and Akinrele (1970)
Oshikundu	Nonalcoholic beverage	Namibia	*L. plantarum*, *L. lactis* ssp. *lactis*, *Lactobacillus delbrueckii* ssp. *delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus pentosus*, and *Lactobacillus curvatus* ssp. *Cuvatus*	Misihairabgwi and Cheikhyoussef (2017), Mu Ashekele et al. (2012), and Hager et al. (2014)

Oti-oka	Alcoholic beverage	Nigeria	*Lactobacillus fermentum* and *S. cereviseae*	Ogunbanwo and Ogunsanya (2012)
Shakparo Ale	Alcoholic beverage	Brewing Companies	Beer fermenters	Chiba et al. (2012), Najdi Hejazi and Orsat (2017), and Barmeyer et al. (2017)
Togwa	Nonalcoholic beverage	Tanzania	*Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus cellobiosus*, *Weissella confuse* and *Pediococcus pentosaceus*	Oi and Kitabatake (2003)
Uji	Nonalcoholic beverage	Kenya, Uganda and Tanzania	*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Pediococcus pentocaceus*, *Lactobacillus paracasei* ssp. *Paracasei*, *Lactobacillus fermentum*, *Lactobacillus cellobiosus* and *Lactobacillus buchneri*	Mbugua (1984) and Masha et al. (1998)
Zoom-koom	Nonalcoholic beverage	Burkina Faso	*Lactobacillus fermentum*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus and Weissella species*	Tapsoba et al. (2017)
Xiao mi jiao	Alcoholic beverage	Taiwan	*Enterococcus faecium*, *Lactobacillus brevis*, *Lactococcus garvieae*, *Lactococcus lactis*, *Leuconostoc carnosum*, *Leuconostoc* (Leu.) *mesenteroides*, *Pediococcus pentosaceus*, *Pediococcus stilesii*, *Weissella cibaria*, *Weissella soli* and *P. pentosaceus*	Chao et al. (2013) and Tsai (2002)

Millet-based products are increasingly used as preventive diets for people suffering from celiac disease; moreover effects of millet foods can be categorized into anti-inflammatory, antidiabetic, cardiovascular disease prevention, and prebiotic and probiotic effects. Study reports have presented evidence that traditional millet-based food products have much slower gastric emptying than modern cereal food products (Cisse et al., 2015). Tylor (2017b) stated that whole grain or slightly refined cereal products contain higher levels of non-starch polysaccharides, lipids, B vitamins, certain minerals, phytate, and notably flavonoid-type phenolics than highly refined grains, irrespective of the species of cereal. Furthermore, there are several food processing factors that greatly influence the grain constituents' characteristics such as starch availability, particle size, and micronutrient availability (Kotásková et al., 2016; Taylor and Duodu, 2015b). However, studies have confirmed that fermenting as methods of processing reduced its tannin content as well as the content of other antinutrients such as trypsin, phytates, and phenolics in millet (Roopashri and Varadaraj, 2015).

Several studies have demonstrated that cereal grains are source of phytochemicals, such as phytic acid, known to lower cholesterol, and phytate to reduce the cancer risk (Mukisa et al., 2017; Roopashri and Varadaraj, 2015). Cereals contain phytate which is 1%–6% of the weight of seed; it is also one of the major nutrient-limiting factors that invariably chelates protein, carbohydrate, and divalent cations (D'Amico and Schoenlechner, 2017). Fermentation is a way to enable activation of inherent phytase in cereal substrates. Roopashri and Varadaraj (2015) explored widely the functionality of phytase of *Saccharomyces cerevisiae* MTCC 5421 to lower inherent phytate in selected cereal flours and wheat/pearl millet-based fermented foods with selected probiotic attribute; and the results showed complete reduction of phytate. It is said that phytate is known to dephosphorylate into lower phosphorylated products and then hopefully even a minimum level of dephosphorylation of phytate can benefit mineral absorption (Brnić et al., 2017). Traditional processing of millet as well as contemporary methods of preparation are ways of adding values, diversifying and bring convenient foods with significant decrease of levels of phytic acid (Efiuvwevwere and Akoma, 1997; Thathola and Srivastava, 2002). Phytic acid and amylase inhibitors were completely eliminated in millet when soaked, debranned, and germinated prior to fermentation. Authors reported that fermentation of processed pearl millet grains leads to significant reduction in antinutritional factors of the grains accompanied by significant improvement in the protein digestibility (Brinch-Pedersen et al., 2017; Buddrick et al., 2014; Eltayeb et al., 2016).

Folate (vitamin B9), also called folic acid, is crucial for proper brain function and plays an important role in mental and emotional health. Folate is a water-soluble micronutrient which is essential for all living organisms. Folate is essential to the human body as it is involved in DNA replication and cell division (Korhola et al., 2014). In particular, folate acts as coenzyme in single-carbon metabolism and is involved in the methylations and formylations that occur as part of nucleotide biosynthesis (Greppi et al., 2017a). Fermentation represents one of the natural possibilities to synthetic folate in foods enrichment. Although, probiotic such as *Bifidobacterium* and *Lactobacilli*, plants and yeasts are known to produce folate, many other living organisms, including human cells, have no ability to produce folate on their own and rely on exogenous sources from the diet (Duodu and Apea-Bah, 2017; Johnson et al., 2017). Several well-studied yeasts species such as *Pichia*, *Saccharomyces*, *Candida*, *Kluyveromyces*, *Cryptococcus*, *Pseudozyma* and *Rhodotorula* are known to produce folate. *Candida glabrata*, for instance, was observed to produce high amount of folate within few days of fermentation. Various millet-based fermented beverages are produced from lactic acid bacteria, which are capable of increasing the folate content (Greppi et al., 2017a; Greppi et al., 2017b; Kariluoto et al., 2006; Korhola et al., 2014). Greppi et al. (2017a) showed that many lactic acid bacteria (LAB) strains isolated from a cereal-based fermented food such as *ben-saalga* are able to produce folate, with differences observed depending on species, strains, growth media, and time of production.

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). Probiotics are live microbial (bacteria and yeasts) food supplements which play an important role in promoting and maintaining human health (Shori, 2015, 2016). Nowadays, live cultures, familiarity linked with fermented foods, may not be considered part of probiotic until they are characterized and associated with their health benefits; knowingly that they are available on the market probiotics in food and dietary supplements. In addition to yogurt, cheese and other types of foods deliver probiotics like fruit/vegetable juices and cereal-based products (Lamsal and Faubion, 2009; Oluwajoba et al., 2013). In addition, cereals have been proven to be suitable carriers to deliver probiotic. Millet-based fermented beverages are good sources of nutrients and microorganisms linked to enhance health benefits (Shori, 2016). Probiotics propose many health benefits such as the reduction in gastrointestinal problems like diarrhea, lowering of serum cholesterol in blood, control of inflammatory diseases, stimulation of the immune system, and improvement in intestinal microbial balance (Singh, 2016; Taylor, 2017a). Since the probiotics are capable of assisting the existing flora, or help repopulate the colon when bacteria levels are reduced by antibiotics, chemotherapy, or disease, thus it is a mechanism for the prevention and treatment of intestinal infections, increase the use of lactose in intolerant individuals, assists in the control of cholesterol serum levels, reduce blood pressure on hypertensive patients, and anticancer properties (Karmally et al., 2005; Wolever, 2013). Millet-based fermented beverages generate fatty acids, vitamins, and many other vital nutrients that improve the body's resistance, and production of antimicrobial substances against pathogens. Bacteriocins produced from millet-based fermented beverages present the great advantage to be derived from harmless substances, antimicrobial peptides generated from fermented foxtail millet showed resistance to digestive enzyme which make their function more important (Amadou et al., 2013b).

Prebiotics are nondigestible food ingredients that beneficially affect the host to improve gut health, enhance immune function, and improve digestion of one, or a limited number of, bacteria in the colon (Angmo et al., 2016; Freire et al., 2017; Hassani et al., 2015). Oligofructose and inulin are plant origin fibers, both are necessary for the main nourishment of good bacteria that reside in the gut. Food ingredients classified as prebiotics must not be hydrolyzed or absorbed in the upper gastrointestinal tract, need to be a selective substrate for one, or a limited number of colonic bacteria, must alter the microbiota in the colon to a healthier composition, and should induce luminal or systematic effects that are beneficial to the host health (Kandylis et al., 2016; Kantachote et al., 2017; Owusu-Kwarteng et al., 2015; Pedersen et al., 2012; Taylor and Duodu, 2015b). Many authors have reported that these fibers are rich in millet-based foods like a number of millet-based fermented beverages.

11.4 Effects of Processing Millet-Based Beverages

11.4.1 Millet in the Industry

Millets have good grain qualities suitable for processing. Processing of the grain for many end uses involves primary (wetting, dehulling, and milling) and secondary (fermentation, malting, extrusion, flaking, popping, and roasting) operations. Being a staple food and consumed at household levels, processing must be considered at both traditional and industrial levels, involving small-, medium-, and large-scale entrepreneurs (Najdi Hejazi and Orsat, 2017). Dehulling is not favorable to millets due to their small grain size; in addition dehulling followed by milling causes nutrient losses. All the millets can be milled by hand grinding (household level) or machine milling (cottage, small-medium- and large-scale industrial services). Milling is a stage that affects the millet nutritional status and prepared products. Generally, flour products from mechanically milled processes are commercially acceptable and have improved shelf life, whereas the traditionally milled products retain more nutrients (Amadou et al., 2011; Taylor and Duodu, 2015a). Nowadays, emerging principal uses of millet or sorghum as an industrial raw material include production of biscuits and confectionery, beverages, weaning foods, and beer. Grits, flour, and meals from cereals like sorghum, corn, and millet are the common items found in the market.

In the infant weaning food sector, in spite of unlimited potential, progress has been slow, as the installed capacity for industrial malting is limited. A couple of beer brands found in the market have substantial contents of local millet including sorghum and maize. The industries are, however, confronted with a number of problems which tend to diminish product qualities and affect overall utilization. For instance, in the nonalcoholic beverage and weaning food sectors, raw material quality, effect of processing on nutritional status, cost of equipment, varieties among cultivars are the few cited problems that militate against improved utilization of millet in the developing countries (Akoma et al., 2006; Chiba et al., 2012; Kerpes et al., 2017; Najdi Hejazi and Orsat, 2017; Thathola and Srivastava, 2002).

11.4.2 Primary Transformation

Many cereal-based beverage processing follows procedures such as cleaning/washing, steeping in water, drying, milling, sieving, and fermentation. Germination in the unit operation is an important stage which needs greater attention, during this process the hydrolytic enzymes bring changes in the endosperm. Some of the vitamins are also synthesized and the bioavailability of minerals increases. During soaking, the soak water is required to be changed once or twice to prevent the excessive growth of microorganisms and also to make it free from CO_2 formed during soaking. During germination, it is essential to mix or turn the grains to provide good aeration to facilitate better germination. To stop the germination process, the grains are dried either in the sun or mechanically dried to a final moisture content of nearly 10%–12% and subsequently the separation of roots and shoots is accomplished by various traditional and modern methods by giving a mild rubbing or abrasion to the grain mass. Higher drying temperature may cause parboiling effect and hardening of the grains which may have adverse effect on milling and quality of the malt flour. The germinated grains are then spread out on a mat or other suitable surface and left to dry in the sun (Akoma et al., 2006; Chiba et al., 2012; Kerpes et al., 2017).

Taylor (2017b) reported that grain refining process, which is called dehulling or decortication, was traditionally achieved by hand

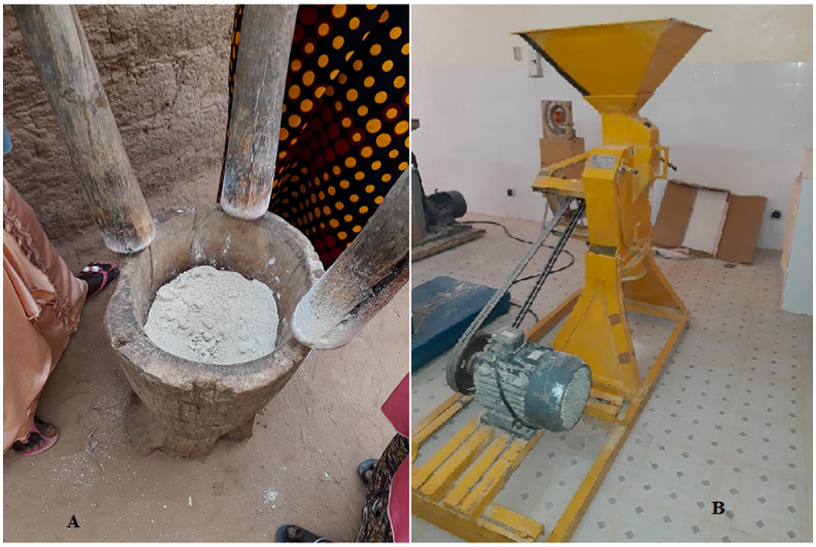

Fig. 11.2 (A) Women from Dan-Gona village (Niger) milling pearl millet grains using wooden mortar and pestles; (B) pearl millet grinder (INRAN, Niger).

pounding using a pestle and mortar or with a simple stone quern type mill, which has two horizontal mill stones, however, today hammer mills (Fig. 11.2) are more commonly used. Despite mechanical decortication, followed by mechanical milling, there are locality where manual milling processes are still used. Fig. 11.2a shows the women from Dan-Gona village (Niger) in the Sahel (Sahara desert margin region) milling millet using mortar and pestles. Traditional millet-based fermented beverages have been reported in many countries, most of the traditional brews are produced in the countryside (Table 11.2) with the exception of some companies and firms (Chao et al., 2013; Taylor, 2017b; Tsai, 2002).

11.4.3 Secondary Transformation

Despite the knowledge related to malting process and importance of sprouted millet consumption, malting process remains a really traditional process in many underdeveloped countries. Traditional malting process involved three main operations: soaking, germination, and drying. The variation of time and state conditions of each unit operation resulted in highly variable quality of the malt or derived products (Chiba et al., 2012). Traditionally, the malt is coarsely ground using a grinding method. Traditionally, the millet malt is used for infant feeding and also to prepare beverages. Total aerobic germs, coliforms, yeast, and filamentous fungi of malt in traditional process are higher than the recommended limits of Codex Alimentarius. Likewise, millets are susceptible to mycotoxin production by fungal growth under certain environmental conditions. Mycotoxin not only threatens consumer health but also is a major threat to the malt quality (Kaur et al., 2014; Limaye et al., 2014). However, product that involves serious fermentation of lactic acid bacteria and cooking to some extent reduces the risk from the pathogens.

Fermentation in food processing is defined as the process of converting carbohydrates to alcohol or organic acids using microorganisms—yeasts or bacteria—under anaerobic conditions. Fermentation usually implies that the action of microorganisms is desired (Wikipedia). Fermentation is a bioprocess that cost efficiently improves the quality, nutritional value, and organoleptic properties of perishable foods. Indeed it modify the carbohydrate contents of foods, synthesize amino acids, improves the availability of B-group vitamins, degrades antinutrients, and thus increases the availability of iron, zinc, and calcium (Amadou et al., 2011; Kantachote et al., 2017).

Moreover, fermentation decreases the starch and long-chain fatty acid contents, leading to an increase in microbial flora, lactic and acetic acid contents, respectively. The total fat content decreases which favorably agrees with total loss in long-chain fatty acid contents. In addition, fermenting microorganisms such as lactic acid bacteria have antimicrobial activities against pathogens and spoilage microbes, and they can enhance the texture, mouthfeel, taste perception, and stability of fermented foods through the production of exopolysaccharides (Amadou et al., 2013c; De Vuyst et al., 2014; Durojaiye et al., 2010), to some extent researches have revealed the high antioxidant activity of fermented millet (Amadou et al., 2013c). This biochemical modification by microorganism contributes directly to many advantageous properties of millet-based fermented beverages.

Modern food processing is based on warranting the quality of food from manufacture to eating plate. One of the ancient technique in processing is fermentation, a process dependent on the production of metabolites by microbial metabolism which can inhibit the growth and survival of undesirable microflora in foodstuffs (Shahidi and Chandrasekara, 2013). Fermentation is a cheaper and economical technique to preserved foods, it increases the acidity and decreases the pH of the substrate, thereby inhibiting many pathogens (Kotásková et al., 2016). Amadou et al. (2016) and Kotásková et al. (2016) added that fermentation in food processing is a mean of detoxification, decreasing cooking times and fuel requirements.

Millet-based fermented beverages are results of the effect of microorganisms or enzymes to cause desirable biochemical changes (Sood et al., 2015; Takahashi et al., 2017). Main millet-based fermented beverages products are listed in Table 11.2, together with the product name, class, location, and strain found. Spontaneous or natural fermentation has been used in less developed countries to ameliorate the nutritional status and preserve foods. Millet-based fermented beverages (alcoholic and nonalcoholic drinks) are mainly prepared for human consumption (Francis et al., 2017). These millet-based fermented beverages are highly appreciated by consumers, and play an important role in their countries of origin and they are also part of the people's culture. These beverages are sometimes attached to peoples' traditions, hospitality as etiquette of some families, and serve to seal the relationships between individuals (Dayakar Rao et al., 2017). The primary activity of the culture in millet-based fermented beverages is to convert carbohydrates to desired metabolites such as alcohol, acetic acid, lactic acid, or CO_2 (Limaye et al., 2014). The cultures used in these fermented beverages, however, also contribute to secondary reactions by improving organoleptic properties and enhancing immune potency (Mosha et al., 1996; Takahashi et al., 2017).

Extrusion technology is one of the ways of transforming ingredients into value-added products. Extrusion is one of the most versatile and well-established industrial processes used in the food industry today. It is being increasingly applied worldwide to materials including snacks, cereals, pastas, textured vegetable proteins, pet foods, animal feeds, instant beverage products, and meat analogues and extenders (Serna-Saldivar, 2016). Millet is a cereal with good amount of starch that can be extruded after making flour and conditioning to required condition. Millet flour or grits exhibit good extrusion characteristics, which could be used to produce fermented beverages.

11.5 Millet-Based Fermented Beverages Products

Even with the absence of gluten in the millets, around the world a wide range of traditional foods and beverage products are produced by them (Taylor, 2017b). Examples are present in Table 11.2. Replacing cereals like wheat, barley, and rye-based foods made with gluten-free grains, including rice, corn, sorghum, millet, amaranth, buck wheat, quinoa, and wild rice may help people adhering to gluten-free diet (Dayakar Rao et al., 2017). As millets are gluten-free grains, they have considerable potential in foods and beverages and can meet the growing demand for gluten-free foods and will be suitable for individuals suffering from celiac disease (Ejeta, 2017; Serna-Saldivar, 2016). As explained, many traditional millet foods are subjected to lactic acid fermentation, thus the acidity is generally similar to that of yogurt. Many millet-based fermented beverages from sub-Samarian region contains tartaric acid, are strongly alkaline due to some additives, and there are many similarities in terms of flavor are found in these traditional millet beverages. Regarding texture, millet products like porridges can be remarkably sticky possibly due to swelling of small granules of starch (Gabaza et al., 2016). In the countries with largest millet production such as in Africa, there is expanding small-scale manufacture of convenience-type versions of traditional millet-based foods (Taylor, 2017b). These products cater for the needs of the growing numbers of urban middle class consumers who are prepared to pay a considerable price premium for convenience and nutrition. Notably, millet is now being used in a number of millet nonproducing countries as an ingredient for the manufacture of instant porridge for infants by many international food companies.

11.5.1 Ben-saalga

Millet-based fermented gruels made in traditional production units can be found in large number in West African countries like Burkina Faso. These gruels are called *ben-saalga.* Traditional cereal-based fermented materials are often used as complementary weaning foods in Africa (Freire et al., 2017; Shori, 2016). Processing comprises washing and soaking of the grains (pearl millet), grinding, kneading, sieving, settling, and cooking and is performed under natural conditions in the yard of the traditional production unit, except for the grinding step which is not performed by the producer himself but in small community milling units. The daily quantity of millet usually transformed into *ben-saalga* using traditional production unit is around 6.8 kg. Usually aromatic ingredients such as ginger, black pepper, pepper, and mint are added in small quantity depending on the traditional producer of *ben-saalga* (Muyanja et al., 2003; Tou et al., 2006). Cooking is the final step of millet processing into gruels (*ben-saalga*). Firstly, after settling step the supernatant is collected and heated to boiling point for 40 min, afterward, the supernatant is added to the paste and boiled for 7 min. Tou et al. (2006) reported that the sour gruel *ben-saalga* a product of cooked sour from paste had inadequate nutritional characteristics with respect to infants' and young children's requirements.

11.5.2 Boza

Historically, beer is supposed to have originated from *boza*, a drink considered 8000–9000 years old. Although the alcohol and acid contents of *boza* was not known at that time, *boza* was recorded in clay tablets as a stimulant and also as a medicine. Its consumption was initially widespread in the Islamic countries, but production was prohibited in the 18th century because of its high alcohol content. It is still produced and consumed widely in Anatolia, South Russia, East European countries, Middle East, and Northern Persia (Evliya, 1990). In Egypt, the traditional way of producing *bouza* (*boza*) beverage still exists, likewise in South African Republic, *boza* production has become an important section of the beverage industry. *Boza* and similar beverages are produced with different recipes and methods in various countries. *Boza* is made by adding clean water to millet and sugar followed by fermentation. Both sweet and sour *boza* depend on its acid content (Arici and Daglioglu, 2002). The steps for *boza* production can be summarized as (i) preparation of the raw materials, (ii) boiling, (iii) cooling and straining, (iv) sugar addition, and (v) fermentation (Fig. 11.3). Studies reported that *boza* is a fermented healthy and nutritious beverage due to its lactic acid content with advantage of improving digestion and intestinal flora. Hancioğlu and Karapinar (1997) isolated and identified eight strains of lactic acid bacteria of *Lactobacillus* and *Leuconostoc genera*, and two yeast strains of *Saccharomyces* from *boza* produced in Turkey; however, some studies reported eleven strains both from lactic acid bacteria and yeast (Arici and Daglioglu, 2002).

11.5.3 Burukutu

In many countries millet has been used successfully as substitute for barley, for instance, grains such as finger millet has been used in

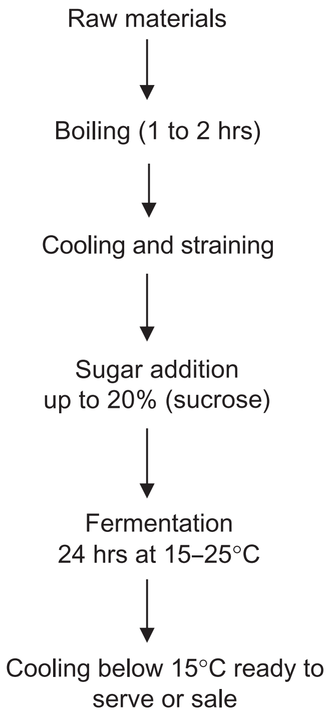

Fig. 11.3 *Boza* production scheme (Arici and Daglioglu, 2002).

the sub-Saharan Africa and India as a major ingredient in the local production of malt (Oguntoyinbo and Narbad, 2012). Traditionally, Africa beers differ from the western beer; they are often sour, less carbonated, and have no hops. They are consumed unrefined, including unfermented substrates and microorganisms (Solange et al., 2014). *Pito* and *burukutu* are brewed concurrently by fermenting germinated or malted millet or mixture of other cereals. *Pito* or *burukutu* is a brownish colored suspension/liquor (Iwuoha and Eke, 1996; Ogunbanwo et al., 2013). *Burukutu*, a popular alcoholic drink among indigenes of sub-Saharan Africa, serves as a source of alcohol for those who lack financial means to patronize refined brew like beer and other foreign or imported drinks. Oluwajoba et al. (2013) produced *burukutu* with combined starter cultures of *Lactobacillus fermentum,* and *S. cerevisiae* then compared it with the *burukutu* produced using the single starter cultures of *L. fermentum* 1 or *S. cerevisiae*. Thus, the combined use of *L. fermentum* 1 and *S. cerevisiae* contributed to the highest characteristic taste, aroma, color and overall acceptability of *burukutu* produced.

11.5.4 Bushera

Western highlands of Uganda is recognized for its traditional beverage *bushera* made from sorghum and millet which are their major staple and commercial crops. The product is popular among young children and adults. To make *bushera* millet or sorghum is mixed with boiling water, cooled to room temperature, followed the addition of germinated sorghum or millet flour and mixed. The mixture is allowed to ferment at ambient temperature for 1–6 days. The lactic acid bacteria isolated from *bushera* comprises five genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus* and *Streptococcus*. *Lactobacillus brevis* was often isolated compared to other species (Kandylis et al., 2016; Muyanja et al., 2003).

11.5.5 Doro

In countries such as Zimbabwe, millet malt is preferred for the traditional brewing process for producing alcoholic beverages. This traditional brew is known as *doro* (Gadaga et al., 1999). *Doro* is brewed for important social and cultural gatherings as well as for generating income. Examples of the social gatherings include when neighboring households come to help with tasks such as weeding and reaping of crops; weddings; celebrations of success; and traditional religious ceremonies such as praying for rain and communicating with ancestors (Cisse et al., 2015). The brewing process described by Benhura and Chingombe (1989) involves preparing *masvusvu* beverage (a sweet drink produced traditionally from malted finger millet in many villages of Zimbabwe) in the same way as *mangisi*. The *masvusvu* is cooled, diluted in clay pots, and allowed to ferment at room temperature for about 2 days. On the third day, the soured product called *mhanga* is boiled for 3–5 h, reducing the original volume by a quarter in the process. The boiled *mhanga* is allowed to stand overnight after which more malt flour is added. Typically, the amount of malt added is about half the quantity used at the starting of the brewing process. On the sixth day some *masvusvu*, two to three times more than the amount cooked on the first day, is prepared and allowed to cool. In the meantime, a small portion of the *mhanga* is strained and kept separately. The straining, the rest of the unstrained *mhanga*, and the fresh portion of cooled *masvusvu* are all mixed together with water to make *biti*. The mixture is fermented for about 2 h and the resulting product which is called madirwa is then strained, mixed with the previously strained *mhanga* and left to ferment overnight. The *doro* is served in an active fermenting state the following morning. The fermentation process takes 5–7 days depending on ambient temperature (Gadaga et al., 1999; Benhura and Chingombe, 1989).

11.5.6 Fura

Sahel is a semiarid and arid areas of Africa, north of the equator; the staple food is *fura* and it is made from millet flour (Duodu and Apea-Bah, 2017). Durojaiye et al. (2010) and Inyang and Idoko (2006) reported comprehensive ways of making *fura* using millet (Fig. 11.4). Millet grain (500 g) was slightly moistened with water (50 mL) and ground in a locally fabricated disc attrition mill. The hull was removed from the grain by winnowing after sun drying, and the grain was ground into flour and sieved through a 0.5-mm opening. The pearl millet flour (250 g) was mixed with powdered black pepper (0.6 g), powdered ginger (3.35 g), and 175 mL of water (95°C) in a mortar. The mixture was kneaded into dough using a wooding pestle, then hand molded into balls, followed by boiling for 30 min at atmospheric pressure. Then the balls were kneaded again in a mortar while still hot, till slightly elastic smooth mass was obtained. The dough was molded into 50 g small balls. The *fura* stiff dough is now reconstituted into liquid consistency using sour milk and consumed generally as afternoon meal balls (Jideani et al., 2010; Pedersen et al., 2012). The strains of lactic acid bacteria and yeasts were detected in *fura* samples. *Enterobacter*, *bacillus*, *Staphylococcus*, *Fusarium culmorum*, *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus Parasiticus*, and *Mucor racemosus* (Inyang and Idoko, 2006; Jideani et al., 2010).

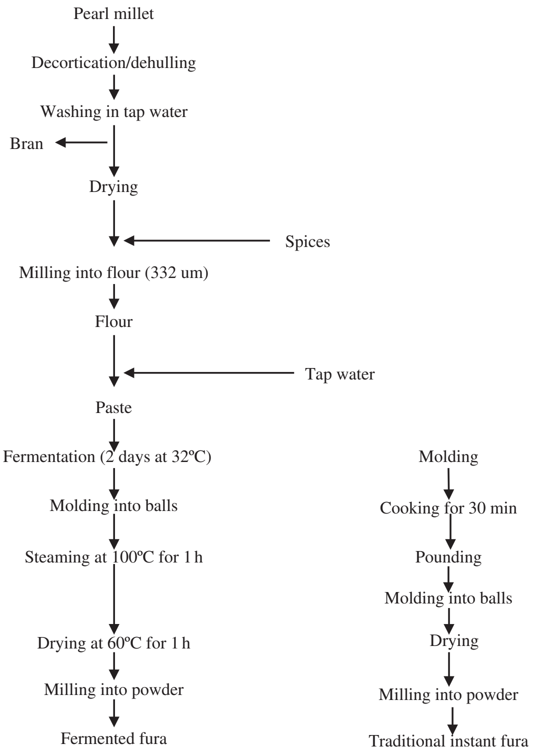

Fig. 11.4 Production of traditional and fermented instant *Fura* (Inyang and Zakari, 2008).

11.5.7 Jandh

The diversified ethnic communities, culture, and heritage dictate various methods of food preparation and the consumption pattern. Traditionally fermented foods are mostly specific to certain geographic regions and also to particular communities likewise *Jandh* (beer type) is one of the major traditional alcoholic products of Nepal. It is a slightly acidic and sweet alcoholic beverage (Dahal et al., 2005). *Jandh* is a fermentation product of finger millet known as *Kodo* or *Marua*. The finger millet seeds are sometimes accompanied with a small quantity of corn or wheat grains (43). *Jandh* is being prepared as follows: the millet seeds are softened through the steam and then spread on leaves, preferably banana leaves. A thorough mixture of boiled *Murcha*, the starter culture, and cooled seeds are piled in a heap and kept for 24 h at ambient temperature. The product is then usually placed in an earthen pot covered with leaves and straw, whereas, in urban areas, the seeds are allowed to ferment in polyethylene bags. If air leaks into the fermentation substrate, which sometimes occurs when the pots are not kept airtight, the product becomes sour (Dahal et al., 2005). After fermentation, the millet seeds are kneaded to remove the coats, then the grits are placed in bamboo vessels either cold or hot. The beverage is ready to drink 10 min later often using a bamboo straw. The liquor is appreciated due to its good tonic, especially for postnatal women (Dayakar Rao et al., 2017).

11.5.8 Kodo ko jaanr

Kodo ko jaanr is the most common fermented alcoholic beverage prepared from dry seeds of finger millet, locally called *kodo* in the Eastern Himalayan regions of the Darjeeling hills and Sikkim in India, Nepal, and Bhutan. According to the traditional method the *kodo ko jaanr* is prepared by drying finger millet seeds, cleaning then washing and cooking for about 30 min in an open cooker, excess water is drained off and the cooked seeds are spread on a mat made up of bamboo for cooling. About 2% of dry powdered *marcha*, a mixed starter culture (Tamang et al., 1996), is sprinkled over cooked seeds, mixed thoroughly and packed in a bamboo basket lined with fresh fern, or banana leaves, then covered with sackcloths, and kept for 2–4 days at room temperature for saccharification. During saccharification a sweet aroma is emitted and the saccharified mass of finger millets is transferred to an earthen pot or to specially made bamboo basket called a septu and made airtight and fermented for 3–4 days during summer and 5–7 days in winter at room temperature (Fig. 11.5). The yeasts and lactic acid bacteria population were detected in *kodo ko jaanr* samples. Yeasts consisted of *Pichia anomala*, *S. cerevisiae*, *C. glabrata*, *Saccharomycopsis fibuligera*, and lactic acid bacteria consisted of *Pediococcus pentosaceus* and *Lactobacillus bifermentans* in *kodo ko jaanr* samples. Good quality of *Kodo ko jaanr* has sweet taste with mild alcoholic flavor. *Kodo ko jaanr* is consumed in an unique way in the Eastern Himalayan regions of Nepal, India, Bhutan, and China (Tibet) (Sanjana Reddy, 2017; Sood et al., 2015; Thathola and Srivastava, 2002).

11.5.9 Koko

Koko is a millet porridge products consumed daily by many people in West-Africa during lunch or in-between meal. *Koko* steps of production constitute overnight steeping, discarding of steep water, and wet milling of the millet grains together with spices (ginger, chili pepper, black pepper, and cloves), addition of water to the milled materials to make it thick slurry. The process is followed by sieving of the slurry, fermentation and sedimentation for 2–3 h, then decanting liquid top layer, boiling of top layer for 1–2 h, and finally addition of sediment to bottom layer until desired consistency obtained. It is a practice of locals to start the process in the evening by steeping the millet grains and the final product is ready for consumption around noon in the

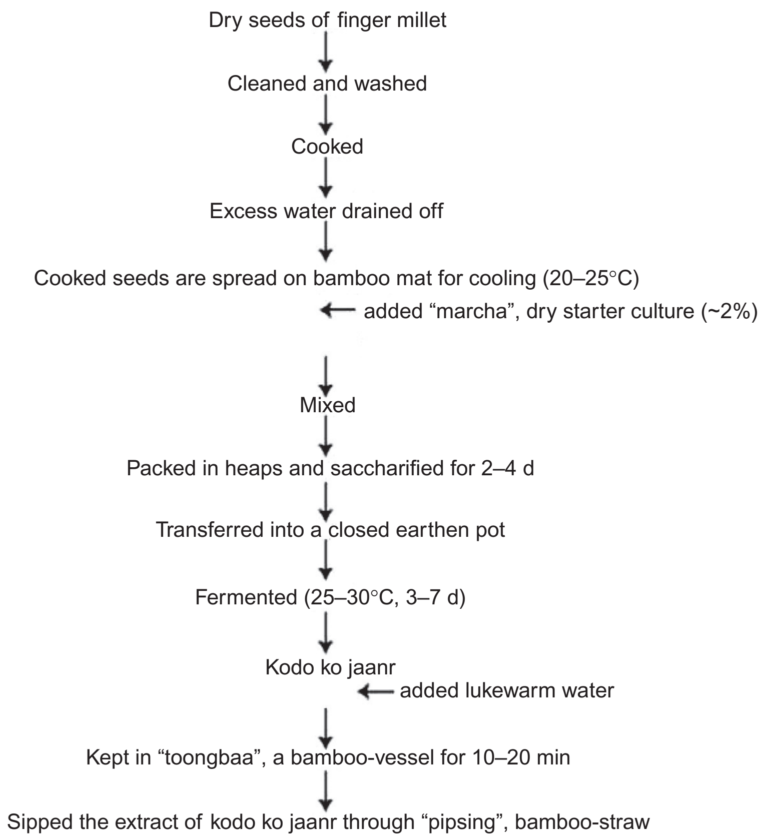
Dry seeds of finger millet
Cleaned and washed
Cooked
Excess water drained off
Cooked seeds are spread on bamboo mat for cooling (20–25°C)
added "marcha", dry starter culture (~2%)
Mixed
Packed in heaps and saccharified for 2–4 d
Transferred into a closed earthen pot
Fermented (25–30°C, 3–7 d)
Kodo ko jaanr
added lukewarm water
Kept in "toongbaa", a bamboo-vessel for 10–20 min
Sipped the extract of kodo ko jaanr through "pipsing", bamboo-straw

Fig. 11.5 Flow sheet of *kodo ko jaanr* preparation (Thapa and Tamang, 2004).

following day. The product is marketed in form of porridge in plastic bags or bowls and is normally consumed sweetened. The dominating strains in *koko* are *Weisella confusa* and *L. fermentum*, followed by *Lactobacillus salivarius* and *Pediococcus* spp. (Geetha, 2013; Lei and Jakobsen, 2004).

11.5.10 Koozh

Koozh is prepared in an outdoor traditional kitchen common in rural India, using traditional methods that have been followed for generations. A flow chart of the typical process is depicted in Fig. 11.6. The millet flour is made into slurry with water by hand mixing on the first day and left to ferment overnight (12–15 h); on the second day, broken rice (20% by weight of millet) is cooked in excess water, into which the fermented (12–15 h) millet slurry is mixed, stirred, and cooked to make a thick porridge. The fermentation of this porridge overnight (24 h) results in *kali*, a semisolid porridge to which the required amount of potable water is added (1:6 w/v) and hand mixed with salt

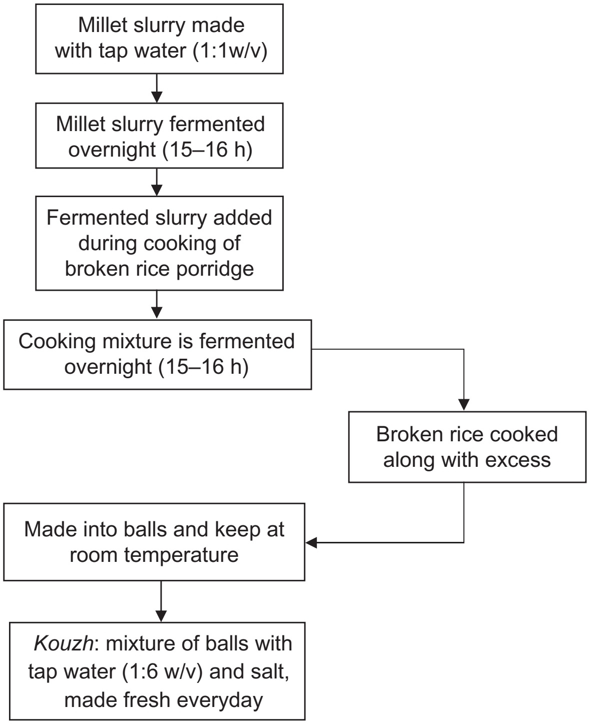
Millet slurry made with tap water (1:1w/v)
Millet slurry fermented overnight (15–16 h)
Fermented slurry added during cooking of broken rice porridge
Cooking mixture is fermented overnight (15–16 h)
Broken rice cooked along with excess
Made into balls and keep at room temperature
Kouzh: mixture of balls with tap water (1:6 w/v) and salt, made fresh everyday

Fig. 11.6 Indigenous preparation of *koozh* from finger millet (Ilango and Antony, 2014).

to prepare *koozh*. The product may be further mixed with yoghurt to give a thin porridge consistency. The end product has a characteristic fermented flavor as a result of the microbial succession, which develops complex flavors. While *kali* has a shelf life of approximately 1 week at room temperature (25–30°C), *koozh* has low shelf life and is usually consumed within about 12 h of preparation (Angmo et al., 2016; Jeong et al., 2014). The second fermentation after cooking of the millet in *koozh* preparation makes it an excellent source of live bacteria, while its storage and service may allow contamination that might improve its probiotics content (Amadou et al., 2016; Kantachote et al., 2017). In all the samples lactic acid bacteria were found to be dominant and yeast counts were comparatively lower in *koozh* (Ilango and Antony, 2014). The microbial quality of *koozh* is considered healthy for its nutritional content, sold as a street food as product varies from locality to locality; the lactic acid bacteria and yeasts also vary.

11.5.11 Kunun-zaki

Sweet Kunun or kunun-zaki is a fermented nonalcoholic drink cereal beverage. It is a well-known drink in the Sahel region such as Northern Nigeria, Tchad, and Niger. It is essentially a home-based industry and at present, no large-scale factory production is found. Its sweet-sour taste with refreshing quality as well as the creamy or milky appearance and also flowing consistency makes it popular. *Kunun-zaki* beverage is basically made from millet which is consumed few hours after it is produced. Agarry et al. (2010) described a typical process for the manufacture of *Kunun-zaki* (Fig. 11.7), cleaned and washed dehulled millet (500 g) are weighed and steeped in 1 L water at ambient temperature for 48 h, then the procedure is repeated four times. This is followed by steeping, milling, and addition of small quantity of spices (ginger, cloves, red pepper, and black pepper). The slurry is sieved,

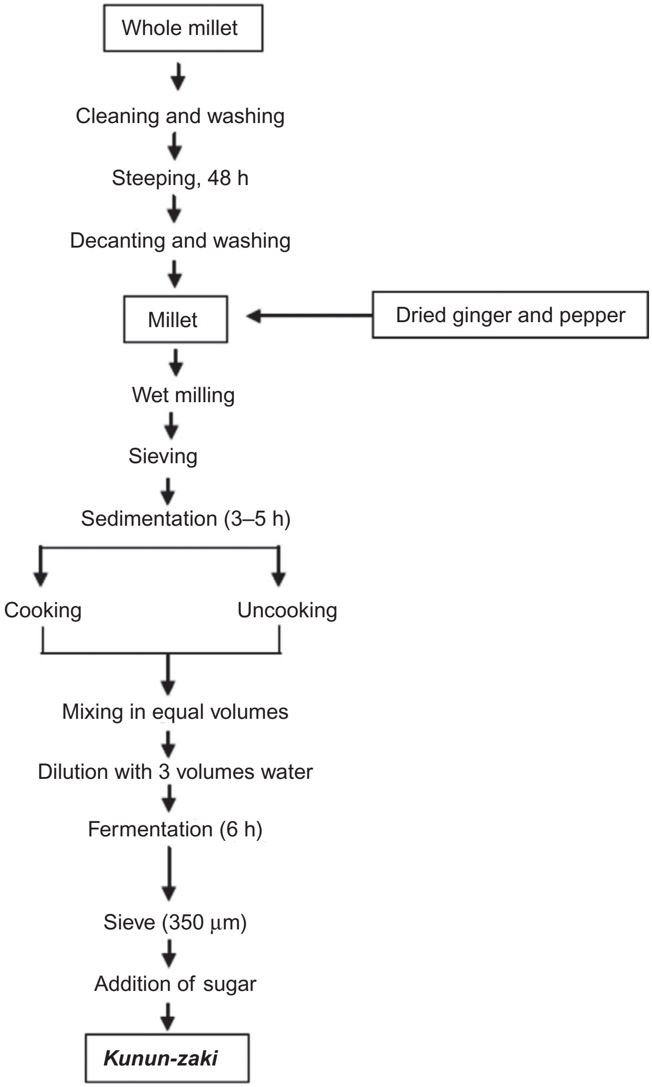

Fig. 11.7 Traditional production of *Kunun-zaki* (Agarry et al., 2010).

aseptically until all the starch is extracted, separated with discarded shaft. The supernatant decanted from settled filtrate and the sediment is divided into two parts (ratio 3:2). The larger portion is cooked by adding boiled water (0.5 L) for 5 min while 0.5 L cold water is added to the second part. Later both slurries are thoroughly mixed and allowed to ferment for 6 h at room temperature then sieved, and sweetened with granulated sugar (50 g). *Kunun-chamia* is another form of *kunun-zaki* but fortified with tamarind extract (Agarry et al., 2010; Amadou et al., 2011; Solange et al., 2014). Microorganism content assessment of *kunun-zaki* sample showed growth of microorganisms belonging to the *L. fermentum, Lactobacillus leichmannii*, *Lactobacillus plantarum*, *P. pentosaceus*, *Lactobacillus pentosus*, *Lactobacillus cellbiosus*, *Leuconostoc mesenteroides*, *Candida mycoderma*, and *S. cerevisiae* (Agarry et al., 2010; Solange et al., 2014).

11.5.12 Madua Apong

The Indian indigenous fermented beverages *Madua Apong* is prepared by taking the cleaned, roasted (30 min) millet grains after soaking for about 3 h, then cooking for 10–5 min. Aseptically the product is cooled after which the cooked millet is mixed with starter culture. The mixture is kept for fermentation for 6 days at 30°C. The filtrate (*Madua Apong*) is collected after sufficient alcohol production by slowly spraying boiled clean water over the mixture at rate of 2 L/h (Sanjana Reddy, 2017; Shrivastava et al., 2015).

11.5.13 Malwa/Ajon

The Ugandans valued a lot their *Malwa* a nonalcoholic fermented beverage due to its flavor, taste, and aroma. *Malwa* is a household level product in Eastern and Northeastern region of Uganda (Muyanja et al., 2003; Solange et al., 2014). The product is consumed in clay pot and while being diluted with hot water using locally made straws. Prior to germination (1–2 days) the clean finger millet grains are soaked in gunny bags overnight (12 h) followed by sun drying (1–2 days) then hammer milled to obtain green malt flour. A stiff dough is made by mixing the non-germinated millet flour with little water, then placed in plastic sheets and buried in the soil to undergo solid-state fermentation (10–14 days) which improves its organoleptic properties. The sour dough is roasted (72°C) on cut metallic drums under open fire with continuous stirring to avoid off-flavor, then sun dried (1–2 days). The process is continued by adding water to the mixture and mashed by boiling. Then mixture is cooled at room temperature prior to adding green malt followed by spontaneous fermentation (2–4 days). The following day some more green malt is added to accelerate the process of fermentation; thus, the *malwa* is obtained (Fig. 11.8).

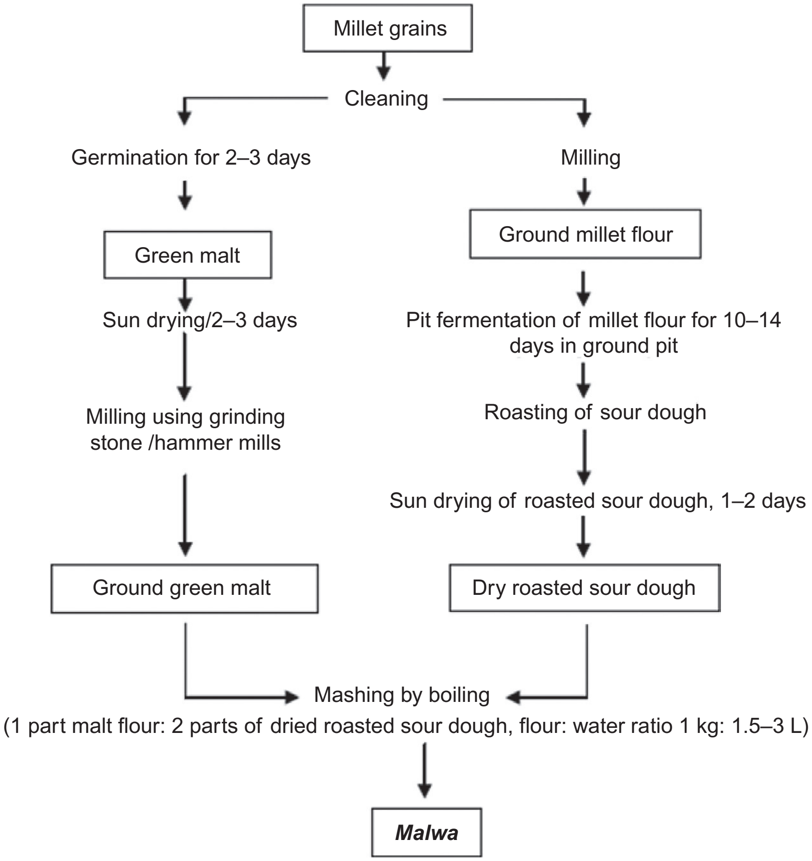
Millet grains
Cleaning
Germination for 2–3 days
Milling
Green malt
Ground millet flour
Sun drying/2–3 days
Pit fermentation of millet flour for 10–14 days in ground pit
Milling using grinding stone /hammer mills
Roasting of sour dough
Sun drying of roasted sour dough, 1–2 days
Ground green malt
Dry roasted sour dough
Mashing by boiling
(1 part malt flour: 2 parts of dried roasted sour dough, flour: water ratio 1 kg: 1.5–3 L)
Malwa

Fig. 11.8 Traditional preparation of *malwa* (Muyanja et al., 2003).

11.5.14 Mangisi

Fermented beverages constitute an important part of the diet of traditional African rural homes served as inebriating drinks, in social functions as well as weaning foods to their role such as marriage, naming, and rain making ceremonies (Vieira-Dalodé et al., 2008) (Misihairabgwi and Cheikhyoussef, 2017). A sweet-sour *Mangisi* beverage is made from the natural fermentation of millet mash (Gadaga et al., 1999). Preparation varies from one region to another of sub-Saharan Africa such as Zimbabwe, Uganda, etc. In one variation, finger millet is malted then milled and the flour is mixed with water. The mixture is heated slowly for 80 min until almost boiling. The resulting product is the mash (*masvusvu*) which is cooled, diluted, strained, and allowed to stand for few hours during which spontaneous fermentation takes place to give *mangisi* (Gadaga et al., 1999). The microorganisms responsible for fermentation are thought to come from

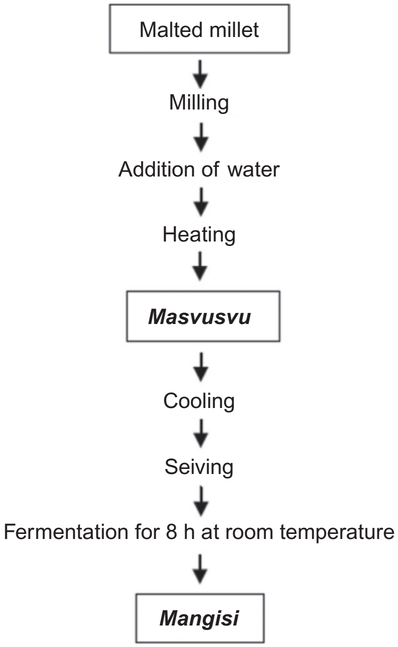

Fig. 11.9 Traditional preparation of *masvusvu* and *mangisi* (Zvauya et al., 1997).

the utensils, fermentation vessel, and include those organisms from the malt flour that survive cooking. Another variation involves malting the finger millet, milling, mixing the flour with water, and boiling the mixture for 1–2 h. The *masvusvu* is cooled, diluted, and allowed to settle for the night. On the following day more malted flour is added to the mixture then allowed to ferment until the third day when the coarse solids are strained off and the fermenting mixture poured into the fermentation vessel. The *mangisi* is ready for consumption later on the same day (Benhura and Chingombe, 1989). Gadaga et al. (1999) reported that the product contain more alcohol due to the extra malt added to the brew on the following day which could have served as an additional source of inoculum and also longer time of fermentation. The microorganisms responsible for fermentation are thought to come from the utensils, fermentation vessel, and include those organisms from the malt flour that survive cooking (Fig. 11.9).

11.5.15 Ogi

It is a porridge made from fermented millet, maize, or sorghum paste or cake in West Africa. Gelatinized *ogi* also known as pap is mostly used as infant weaning food as well as breakfast meal for many adults in the region, which has awesome swell up capacity when mixed with either cold or hot water. Fermentation bring benefits if good manufacturing process is respected, that is, for instance, to inhibit growth

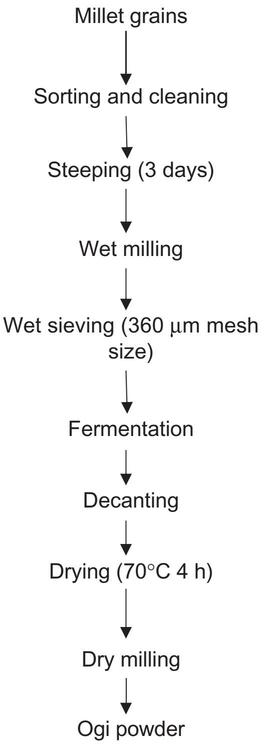

Fig. 11.10 Flow chart of the production of fermented millet *Ogi* powder.

of pathogens through the fermentation process (Abdalbasit et al., 2017). Both industrialized and traditional methods for manufacturing *ogi* have been reported by Taylor and Awika (2017). The lactic acid bacterium *L. plantarum*, the yeasts *C. mycoderma*, *S. cerevisiae* and *Rhodotorula*, and molds *Cephalosporium*, *Fusarium aspergillus*, and *Penicillium* are the most isolated microrganisms and responsible for the fermentation and nutritional improvement of *ogi* (Akinrele, 1970). Odunfa and Adeyele (1985) determined that *L. plantarum* was the predominant organism in this fermentation (Inyang and Idoko, 2006). A flow chart of the production of fermented millet Ogi powder is depicted in Fig. 11.10. The aroma of this product comes mostly from the produced lactic acid and acetic acids, respectively. Moreover, some traces of formic acid are also revealed in *ogi*, characteristic of the product that improved its nutritional attributes (Inyang, and Idoko, 2006; Taylor and Awika, 2017). Mild sour *ogi* with light color is reported to be preferred (Inyang, and Idoko, 2006; Akinrele, 1970).

11.5.16 Oshikundu

Oshikundu is a popular nonalcoholic fermented beverage in the North-central and Kavango regions of Namibia which is brewed from

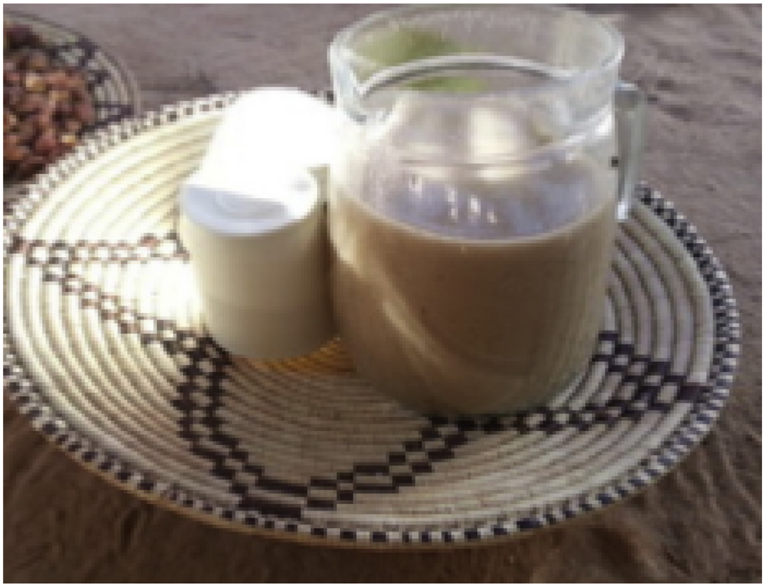

Fig. 11.11 Ready to drink *oshikundu* in a jug (Misihairabgwi and Cheikhyoussef, 2017).

pearl millet or sorghum. Culturally, serving of *oshikundu* (Fig. 11.11) to visitors is considered as a token of welcome and hospitality, and the beverage is produced as a part of the traditional initiation of young girls into womanhood (Mu Ashekele et al., 2012; Hager et al., 2014). The first stage of traditional *oshikundu* production involves the addition of boiled water to pearl millet flour and stirring in one procedure and addition of warm water to pearl millet flour and sorghum malt followed by stirring in the second procedure. The mixture is cooled at ambient temperature after which sorghum malt is added to the mixture and thoroughly stirred in the first procedure. Pearl millet bran can be added optionally at this stage in either the first or second procedure. Water is then added in the mixture to the desired volume and consistency. Previously fermented *oshikundu* is added for backslopping, furthermore, the mixture is allowed to ferment for 4–6 h in a shaded area (Mu Ashekele et al., 2012). Six predominant lactic acid bacteria were found to involve in the *oshikundu* process: *L. plantarum*, *L. lactis* ssp. *lactis*, *L. delbrueckii* ssp. *delbrueckii*, *L. fermentum*, *L. pentosus*, and *Lactobacillus curvatus* ssp. *cuvatus* (Misihairabgwi and Cheikhyoussef, 2017).

11.5.17 Oti-oka

Oti-oka is an indigenous alcoholic beverage made from sorghum and pearl millet with a good flavor, sweet, slightly sour with a brownish-opaque color, a pleasant taste on the third day of fermentation (Oguntoyinbo and Narbad, 2012; Solange et al., 2014). Fig. 11.12

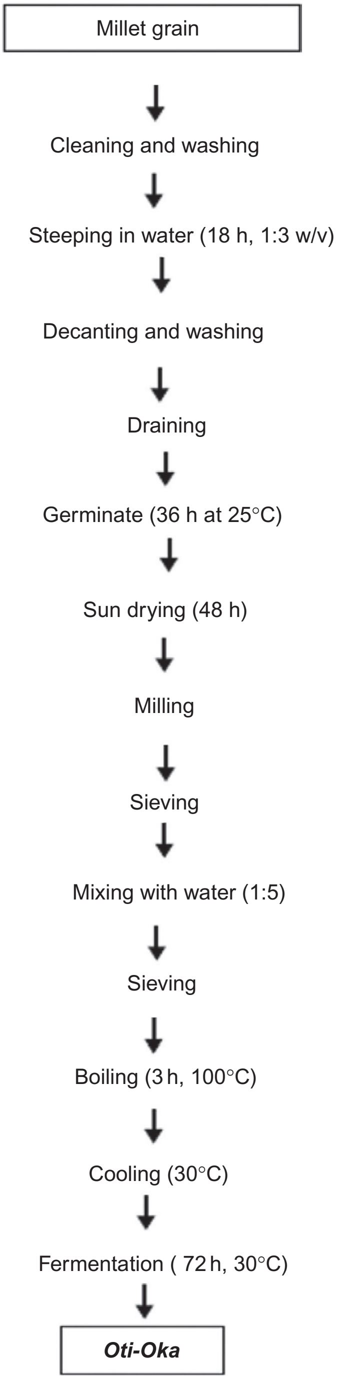

Fig. 11.12 Production of *oti-oka* (Ogunbanwo and Ogunsanya, 2012).

shows how this Southwest Nigerian traditional drink is made, as reported by Ogunbanwo and Ogunsanya (2012). The process start with cleaning, steeping for 18 h after which they are rinsed thrice and the millet grains drained, followed by germination, sun dried for 48 h, grounded and sieved. An aliquot is mixed with water (1:5), sieved with muslin cloth, and allowed to boil for 3 h, then cooled to 30°C and fermented naturally at 30°C for 72 h. Isolated yeast and lactic acid bacteria, *L. fermentum* and *S. cerevisiae*, in *oti-oka* with health benefits like other millet-based fermented beverages were used for fermentation with pearl millet as substrate (Duodu and Apea-Bah, 2017; Solange et al., 2014).

11.5.18 Shakparo Ale

When the malt from cereals sorghum or millet was mashed, both cereals produced wide spectra of substrates (sugars and amino acids) that are required for yeast fermentation when malted at either lower or higher temperatures. Sorghum has gained wide application in the brewing industry, and has been used extensively in brewing gluten-free beer on industrial scale, this is not the case with millet. The work described here provides novel information regarding the potential of millet for brewing. When both cereals were malted, the results obtained for millet in this study followed patterns similar to those of sorghum. This suggests that millet, in terms of sugars and amino acids, can play a role similar to sorghum in the brewing industry. Furthermore, millet, like sorghum, is a good raw material for brewing gluten-free beer. *Shakparo Ale* is gluten-free beer similar to the West African *Tchapalo*-style beer brewed from sorghum and/or millet. An unfiltered, light, crisp ale with a cider or fruit profile and a dry vinous aftertaste, it is best characterized as an easy-drinking or session beer perfect for summer gatherings. This beer pairs nicely with suya (grilled meat), salads, chicken, fish, and spicier foods (Chiba et al., 2012; Najdi Hejazi and Orsat, 2017; Taylor, 2017b).

11.5.19 Togwa

Togwa is a fermented gruel or beverage prepared either from millet or in combination with other local flour (Amadou et al., 2011; Oi and Kitabatake, 2003); it is usually made from the maize flour and malted finger millet. It is locally consumed by the working people as refreshment and also used as a weaning food (Oi and Kitabatake, 2003; Solange et al., 2014). *Togwa* is also regularly consumed by young children. *Togwa* is made by cooking cereal or cassava flour in clean water, cooling then adding starter culture (old *togwa*) and germinated cereal flour are put in. Fermentation is spontaneous and uncontrolled thus resulting in a product of variable quality (Amadou et al., 2014; Amritha et al., 2018). The fermentation is carried out at ambient temperature and the bacteria isolated from *togwa* included *Lactobacillus cellobiosus*, *L. plantarum*, *L. fermentum*, *L. brevis*, *W. confuse*, and *P. pentosaceus*. All of them were present from the beginning to the end of the fermentation. Majority of the bacteria belonged to the *L. plantarum* group, which dominated at the end of *togwa* fermentation. *L. plantarum* has been identified as the dominant microorganism toward the end of spontaneous lactic acid fermentations (Amadou et al., 2013c; Shori, 2015). *Togwa* is widely produced in Tanzania for use directly as a weaning food or diluted for use as refreshment.

11.5.20 Uji

A lactic-fermented porridge *Uji* is well known in East Africa (Kenya, Uganda, and Tanzania). It is prepared by spontaneous submerged culture of lactic acid fermentation of cereals (maize, finger millet, or sorghum) and cassava flours mixed in different proportions. A backslopping technique of fermentation or inocula are used. A ratio of 1:1 composites of maize plus sorghum, maize plus finger millet, cassava plus finger millet, and cassava plus sorghum are used (Onyango et al., 2004, 2005). *Uji* is popular among rural communities and low-income workers in urban areas of East Africa, referred as *togwa* and *obusera* in Uganda and Tanzania, respectively. Adults consume it as a refreshing drink at any time of the day and children consume *uji* as the principal weaning food (Onyango et al., 2003). *L. plantarum* is the predominant lactobacilli species of an *uji* responsible for the higher acidity and the subsequent sour taste. Other homofermenters are *Pediococcus acidilactici*, *Pediococcus pentocaceus*, and *Lactobacillus paracasei* ssp. *paracasei*; while the important heterofermenters are *L. fermentum*, *L. cellobiosus*, and *Lactobacillus buchneri* (Masha et al., 1998; Mbugua, 1984).

11.5.21 Xiao mi jiao

The Taiwanese millet alcoholic beverage is brewed without distillation and is an important traditional offering during the harvest festival of the indigenous Taiwanese people. As its name implies, millet alcohol was made exclusively from millet (*Setaria italic*) (Owusu-Kwarteng et al., 2012) in ancient times. The millet alcoholic beverages were produced by a tribal unit of Amis in Hualien County in Taiwan. Different indigenous Taiwanese tribes have distinct millet alcohol manufacturing processes. During solid fermentation, the steamed millet is mixed with 0.4% starter made from rice flour and plant materials. After 3 days, a 2.5-fold volume of water is added to initiate liquid fermentation. The mixture is stirred thoroughly once per day for 5 days. One year of fermentation followed by liquid filtration is required to prepare this well-aged millet alcohol. The starters were prepared using traditional method, which lead to the identification of 38 strains belonging to 10 species *L. brevis*, *E. faecium* group, *P. pentosaceus*, *Lactococcus garvieae*, *Lactococcus carnosum*, *Lactococcus lactis*, *L. mesenteroides, Pediococcus stilesii*, *Weissella soli*, and *Weissella cibaria*, and (Chao et al., 2013; Taylor, 2017b; Tsai, 2002).

11.5.22 Zoom-koom

The *zoom-koom* is a nonalcoholic beverage, prepared from millet and rarely from sorghum, and much appreciated by consumers in Burkina Faso. It is produced from shelled cereal or whole grains. Millet and sorghum are the main cereals used as raw materials in the production of *zoom-koom*. The production process of *zoom-koom* includes the following steps: (1) Soaking, after washing the millet or sorghum grains are weighed and soaked in a quantity of water equal to twice the mass of millet or sorghum grains (2:1, w/w). The soaking time is set at 16 h. (2) Grinding, the flavored and aromatization ingredients are added to the soaked grains (millet or sorghum) at the rate of 3 g/100 g for mint and 6 g/100 g for ginger, before the wet grinding. (3) Fermentation, the dough is left to stand for fermentation for 10 h (average time observed in the field). The fermentation was realized at ambient temperature (33–42°C). (4) Filtration, a quantity of water equal to three times the mass of the wet dough was added to the dough. The suspension is filtered by pouring into a muslin bag (mesh ≤0.5 mm) to get a zoom-koom without sugar. The production processes of *zoom-koom* by Tapsoba et al. (2017) have been used to establish the flow diagram of *zoom koom* based on millet grains (Fig. 11.13). *Zoom-koom* is the street food which contains more count of thermotolerants and coliforms bacteria. Tapsoba et al. (2017) had shown, throughout a control fermentation of millet *zoom-koom* with *L. fermentum* strain as a starter, that the lactic fermentation allowed the reduction of enterobacteria counts and kept safe the final product. Though, several investigations have shown the involvement of lactic acid bacteria in African traditional fermented cereal-based beverages such as *zoom-koom*, including *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Weissella* species (Geetha, 2013; Ojokoh and Bello, 2014).

11.5.23 Other Fermented Millet-Based Beverages

Rabadi is a popular traditional fermented beverage of India prepared from a mixture of flour of pearl millet (*Pennisetum typhoideum*) and buttermilk (Roopashri and Varadaraj, 2015). *Mougoudji* is a millet-based beverage from Mali, often enriched with fruit juice. *Gowé*

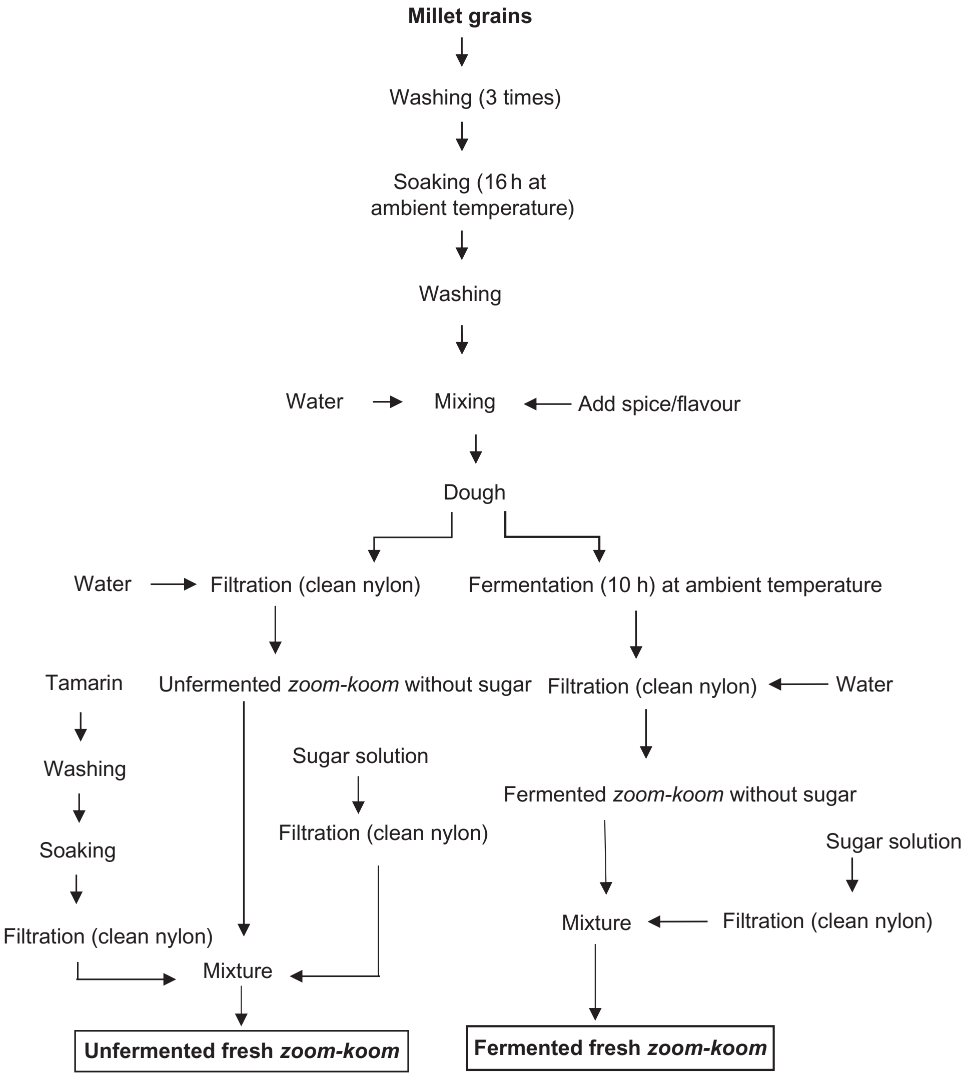

Fig. 11.13 Flow diagram of fresh *zoom-koom* (Tapsoba et al., 2017).

is a nonalcoholic beverage from Benin where millet, maize, sorghum, or millet is gelatinized homogeneously, malted, and fermented then cooked (Solange et al., 2014). *Epwaka* is a pearl millet bran fermented beverage obtained after pounding and sifting pearl millet grains; it is perceived to contain moderate level of alcohol (Misihairabgwi and Cheikhyoussef, 2017). *Okatokele* is commonly brewed among the Oshiwambo speaking people (Namibia), pearl millet flour is mixed with water and sugar then left to ferment in plastic buckets for 8–24 h at room temperature, following which *okatokele* is ready to drink.

Kweete is made from equal parts of maize flour and germinated millet in many parts of Uganda (Mwesigye and Okurut, 1995).

11.6 Conclusion

Millets are still the staple food for millions of poor people in Africa and Asia. Like many other cereals, millets have high carbohydrate, energy contents and nutritious, making them useful components of dietary and nutritional balance in foods. Successful improvement in these attributes would be a crucial to expand the spectrum of applications of millet grains. Thus, this work provides a potential platform for the millet-based fermented beverages; and it can be deduced from this that millet have diverse potentials in brewing and diversity of products. The processing methods, equipment, and conditions need to be completely transformed from traditional to modern, though gradually brewing industries are trying toward perfections of these beverages.

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12

KEFIR: THE CHAMPAGNE OF FERMENTED BEVERAGES

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12.1 Introduction

The kefir drink can be equated to Champagne for several reasons: it dates back a number of centuries; is effervescent with a sparkling mouth-feel; has a unique flavor with a distinct yeasty taste, with yeasts retained in the beverage; contains some ethanol (Duitschaever, 1989; Kwak et al., 1996; Beshkova et al., 2003; Farnworth, 2005; Arslan, 2015; Liger-Belair, 2016); and ideally involves a controlled secondary fermentation in the bottle, the aim of which is maximizing the effervescence and the flavor (Rattray and O'Connell, 2011). Like Champagne, the distinctive flavor stems from a plethora of volatile flavor compounds formed during the fermentation (Farnworth, 2005; Walsh et al., 2016), the most notable of which are diacetyl (or 2,3-butanedione) (Duitschaever, 1989; Rattray and O'Connell, 2011; Walsh et al., 2016), acetaldehyde, and acetoin (Farnworth, 2005; Rattray and O'Connell, 2011). Beshkova et al. (2003) describes the flavor as exotic compared to that of other fermented milk beverages.

Dating back around 2000 years, the somewhat legendary origins of kefir can be traced to the northern Caucasian (also described as the Tibetan or Mongolian) mountains in Central Asia (Nielsen et al., 2014; Rattray and O'Connell, 2011; Arslan, 2015; Rosa et al., 2017), with proteomic evidence proving the existence of kefir in the Early Bronze Age (1980–1450 BC) in Xinjiang, China (Yang et al., 2014; Xie et al., 2016). It remained a closely guarded secret among these tribes and only in the early 1900s were grains acquired by Russian dairy owners, with the details of the acquisition a fascinating tale, as described by Shavit (2008). Nowadays, kefir grains, and the commercial or artisanal beverage produced from it, are found around the globe (Nielsen et al., 2014; Rattray and O'Connell, 2011; Arslan, 2015; Rosa et al., 2017). In Europe it is consumed in many countries (including Bulgaria, Denmark, Greece, Finland, Hungary, Ireland, Italy, Poland,

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Portugal, Spain, and Sweden), while in Eurasia and Asia it is found in Russia, Turkey, China, Hungary, India, Iran, Japan, Malaysia, Taiwan, Thailand, and Tibet, *inter alia*. It is also consumed in the Middle East, North Africa, Canada, the USA, South America (Argentina and Brazil), as well as in South Africa (Koroleva, 1988a, Liu et al., 2002a; Loretan et al., 2003; Otles and Cagindi, 2003; Farnworth, 2005; Shavit, 2008; Aghlara et al., 2009; Nielsen et al., 2014; Rattray and O'Connell, 2011; Arslan, 2015). Large-scale industrial production of kefir is prevalent around the globe, in Australia, Austria, the Czech Republic, France, Germany, Israel, Luxembourg, Norway, Poland, Slovakia, Switzerland (Prado et al., 2015; Rosa et al., 2017) and Russia, where it is the most popular fermented milk beverage of all times (Shavit, 2008).

The global popularity of kefir is also evident from the fact that there are several commercial producers of kefir around the world. These include Lifeway kefir (USA, UK, and Canada) (Prado et al., 2015; Spinler et al., 2016a,b), Bionova (Italy), Latteria Kefir (Austria) (Bertazzo et al., 2016), Evolve Kefir (USA), Wallaby Organic (Australia), and CocoKefir (USA) (Prado et al., 2015). Moreover, in a study of dietary patterns, Norwegian subjects identified kefir in one of the food consumption clusters, with consumption indicated more than three times per week, exceeding their intake of milk (Brustad et al., 2008).

The origin of the name kefir is also cloaked in mystery. It is variously speculated that its origins are Turkic or Slavic, deriving either from "*kef*" or "*keif*" or "*keyif*", the translation of which includes terms like "good feeling," "pleasure," and "well-being," hence alluding to its health-promoting properties and the overall sense of well-being resulting from its consumption (Nielsen et al., 2014; Arslan, 2015; John and Deeseenthum, 2015; Rosa et al., 2017). Nowadays, the traditional beverage is still produced under a variety of names, including kefyr, kephir, kefer, kiaphur, knapon, kepi, and kippi (Sarkar, 2007; Chifiriuc et al., 2011; Rattray and O'Connell, 2011).

Sugary kefir beverages, including various fruit-based fermented beverages, as well as kefir made with substrates that are dairy substitutes are also produced (Liu et al., 2002a; Silva et al., 2009; Puerari et al., 2012; Baù et al., 2013, 2015; Nielsen et al., 2014; Alzate et al., 2016; Gao and Li, 2016; Randazzo et al., 2016). However, while some discussion of these beverages is included, in the interest of simplicity, this chapter focuses on milk-based kefir, with cow's milk as the substrate in particular.

The aim of this chapter was to focus on the unique aspects of kefir as a fermented dairy beverage with recognized probiotic properties. The composition of the grains and the beverage, both in terms of the microbiota and other components, specifically the kefiran, was explored, with a view to highlight their role in the process, the quality of the beverage and the putative health effects. From a process point of view, the role of the culture was emphasized, as well as the challenges and strategies to obtain an absolutely defined or standardized culture and hence final product (beverage). The potential of exploiting advances in culture-independent and OMIC-methods, in other words a systems biology approach to achieve a well-defined culture and process was explored. Food applications of kefir, other than the well-known beverage were also covered.

12.2 Structure, Composition, and Formation of the Kefir Grains

The physical appearance of kefir grains are most frequently described as resembling miniature cauliflower florets (Fig. 12.1A) in texture and color (Liu et al., 2002a; Farnworth, 2005; Gao and Li, 2016; Rosa et al., 2017), but in fact various other manifestations occur, namely popcorn, coral, and cottage cheese with the color ranging from white to creamy yellow (Fig. 12.1B) (Otles and Cagindi, 2003; Farnworth, 2005; Nielsen et al., 2014; Rosa et al., 2017). The grains are also irregular, varying in size from 0.3 to 3.5 cm in diameter (Garrote et al., 1997; Nielsen et al., 2014; Rosa et al., 2017). In our laboratories, it was observed that kefir grains with the typical cauliflower structure (Fig. 12.1A) change to the cottage cheese configuration (Fig. 12.1B) when the grains are inoculated at a concentration of 5%–7%, followed by incubation at elevated temperatures (32–35°C) without stirring and for relatively long periods (3–4 d). Since this format of the kefir culture has a more amorphous structure and a softer texture than the grains, it is more difficult to handle—when strained, a larger proportion of the culture passes through into the fermentate (unpublished results). Hence, it is possible that nonideal process conditions, such as the aforementioned, result in some dissolution or disintegration of the kefiran matrix, leading to the more unstructured manifestation depicted in **Fig.** 12.1B**.**

Kefir grains encompass a complex symbiotic microbial ecosystem, with the constituent organisms embedded in coagulated protein which assembles into groupings with a specific organization within an elastic, jelly-like, yet resilient, and exopolysaccharide (EPS) matrix (Farnworth, 2005; Rodrigues et al., 2005; Vinderola et al., 2006b; Nielsen et al., 2014; Rosa et al., 2017). One aspect of the specific organization described by Golowczyc et al. (2009) is the co-aggregation between LAB (*Lactobacillus kefiri*) and yeast (*Saccharomyces lipolytica*), mediated by bacterial surface-layer proteins (SLPs) and yeast carbohydrates. The ecosystem or symbiotic/symbiont microbial community or consortium (Van Wyk et al., 2011) is also referred to as a symbiont microbiota (Vinderola et al., 2006b). The EPS matrix protects the

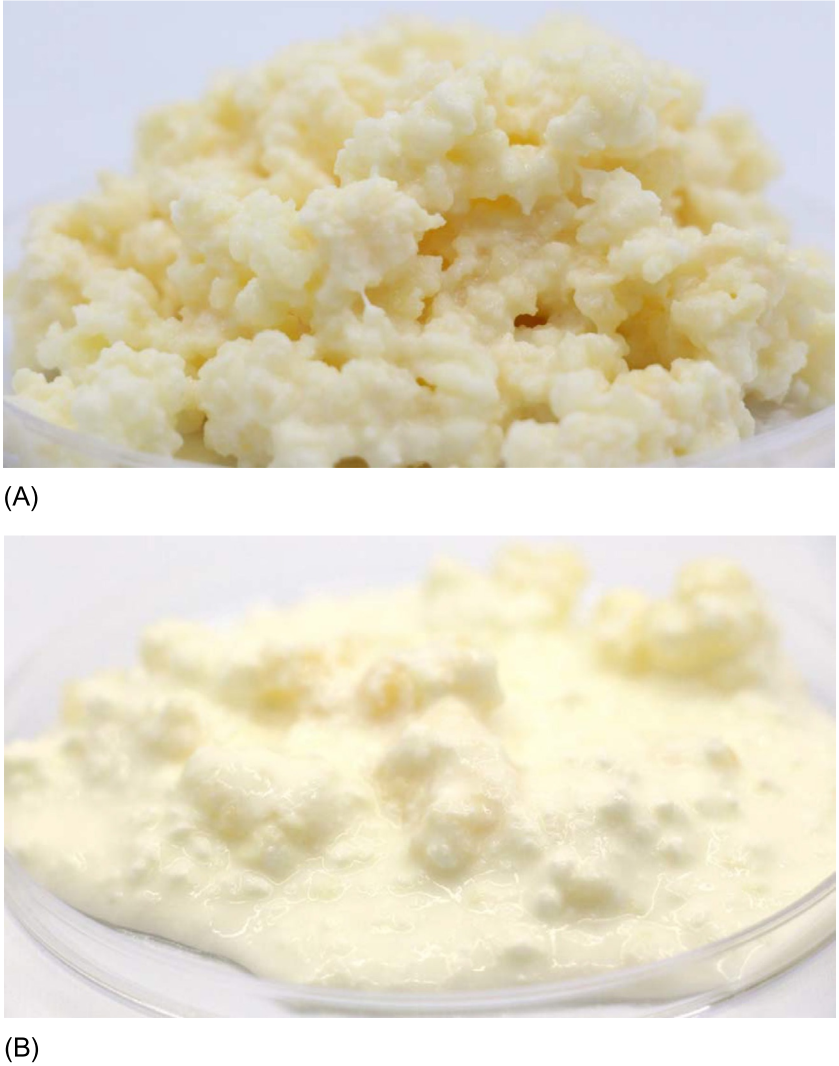

Fig. 12.1 (A) Kefir grains with the typical cauliflower appearance and (B) kefir grains with a cottage cheese appearance.

microbiota within its confines against adverse environmental effects, including heavy metals and desiccation (Badel et al., 2011; Hussain et al., 2017). It is composed of equal quantities of D-glucose and D-galactose, with the major component of this fraction being kefiran, a branched glucogalactan with an apparent molecular weight (M_w) of 1×10^4–1.7×10^6 Da. The main producers of kefiran are *Lactobacillus kefiranofaciens*, *Lb. kefiri* (Liu et al., 2002a; Rimada and Abraham, 2003; Farnworth, 2005; Rimada and Abraham, 2006; Carasi et al., 2014a, 2015;

Piermaria et al., 2016; Rosa et al., 2017; Toscano et al., 2017), and *Lactococcus plantarum* (Gao and Li, 2016). However, other species may also produce kefiran (Kroger, 1993; Rea et al., 1996), and other EPS, such as the high M_w (18.4×10^6–21.9×10^6 Da) EPS produced by *Lactobacillus paracasei* strains (Hamet et al., 2015; Bengoa et al., 2018), the novel EPS with bactericidal activity (*Lb. kefiranofaciens*) (Jeong et al., 2017), two heteropolysaccharides elaborated by *Lb. plantarum* strains, with promising food additive properties (Gangoiti et al., 2017), as well as with antioxidant, antimicrobial and antitumor activity (Wang et al., 2015), and another EPS with high antioxidant capacity (Chen et al., 2015). Kefiran comprises 45% of the dry weight of the grains (Badel et al., 2011; Exarhopoulos et al., 2017). Kefiran production by *Lb. kefiranofaciens* and *Lb. delbrueckii* subsp. *bulgaricus* is stimulated by the presence of *Saccharomyces cerevisiae*, demonstrating another facet of the symbiosis between bacteria and yeasts in the microbiota (Frengova et al., 2002; Rattray and O'Connell, 2011). Kefiran is sometimes incorrectly described as water insoluble (Rea et al., 1996; Farnworth, 2005), but is indeed water soluble (Dailin et al., 2016; Exarhopoulos et al., 2017). Extraction using boiling water yields up to 42% pure EPS (Rodrigues et al., 2005; Rimada and Abraham, 2006; Medrano et al., 2008; Piermaria et al., 2008; Gao and Li, 2016; Rosa et al., 2017) and, in hot water (80°C), 2% pure kefiran forms a viscous solution (Farnworth, 2005; Exarhopoulos et al., 2017). Moreover, kefiran does contribute to the viscosity of the kefir beverage, confirming its water solubility even in cold water/milk (Rosa et al., 2017). The versatility of kefiran is further demonstrated by its ability to form exceptionally thin edible films that are transparent, flexible and shows satisfactory barrier and mechanical properties (Piermaria et al., 2015).

The robustness of the kefir grains and the ability of the microbiota to fully restore its original equilibrium after repeated subculturing of grains whose microbial balance had been disturbed by adverse conditions are attributed to the resilience of the inert polysaccharide-polypeptide matrix, the kefiran in particular. This was demonstrated when kefir grains were subjected to extreme adverse conditions, including homogenization, liquid nitrogen, and frozen storage, resulting in a shrunken appearance and imbalanced microbiota, but after multiple sequential subculturing in milk, they were restored to their former state (Nielsen et al., 2014). The fact that the grains are insoluble in water and ordinary solvents further explains the observed resilience (Kosikowski, 1982), as well as the potential to remain active in perpetuity (Shavit, 2008).

12.2.1 Composition of the Kefir Grains

The fact that the total number of microorganisms isolated from various kefirs is estimated to exceed 300 species emphasizes their complex and diverse composition (Rosa et al., 2017). However, the typical number of organisms constituting the microbial community constituting any individual kefir grain culture ranges between 30 and 100 organisms (Teusink and Molenaar, 2017; Macori and Cotter, 2018). Moreover, the diversity among grain cultures is a function of the origin of the grains, type and composition of the milk, processing method and conditions as well as storage conditions of both the grains and the beverage. The processing conditions of particular importance are: agitation; the inoculum concentration; as well as the fermentation time and temperature (including variations in ambient temperature where fermentation is conducted at “room temperature”). These parameters influence the rate and extent of acidification most directly, hence dictating the microbial composition of the fermented beverage, since rapid acidification may result in feed-back inhibition of organisms (Gao and Li, 2016; Rosa et al., 2017) such as LAB (Rattray and O’Connell, 2011).

The main groups of microorganisms present in kefir grains are: lactobacilli; mesophilic homofermentative lactococci; mesophilic heterofermentative lactococci; lactose-fermenting and nonlactose-fermenting yeasts; filamentous fungi; and acetic acid bacteria (Koroleva, 1988a; Rattray and O’Connell, 2011; Rosa et al., 2017). The symbiotic relationship between the organisms serves to maintain the specific profile in the grains and the beverage, regardless of variations in substrate quality, including microbial contamination due to lack of aseptic handling, or the presence of antibiotics, and other inhibitory agents (Gao and Li, 2016; Rosa et al., 2017). The most striking aspect of the symbiosis is the fact that yeasts (and even the *Acetobacter* spp.) stimulate growth of the LAB in the consortium by producing essential growth factors, notably amino acids and vitamins, while bacterial metabolites, such as lactates, serve as energy sources for the yeasts (Ponomarova et al., 2017; Rosa et al., 2017). *S. cerevisiae* (Ponomarova et al., 2017), *Yarrowia lipolytica*, and *Debaryomyces hansenii* are unable to metabolize lactose (Ponomarova et al., 2017), but utilize lactates, which then also leads to a pH increase, further enhancing growth of LAB by preventing potential feed-back inhibition by the lactic acid (Rattray and O’Connell, 2011). Ponomarova et al. (2017) confirmed this cross-feeding interdependency between *S. cerevisiae* and *Lactococcus lactis* (isolated from kefir). Moreover, their results elucidated the regulatory systems that prompt the secretion of amino acids by the yeast and hence strongly supported the proposal that this secretion is a mechanism to dispose of excess, and potentially toxic levels of, intracellular nitrogen (i.e., nitrogen overflow). In turn, the secreted amino acids are essential growth-limiting nutrients, that is, vital toward supporting the growth of the more fastidious LAB. Thus an instantaneous and stable symbiotic relationship is created, which can also be classified as true mutualism.

The bulk of the microbial biomass consists of *Lactobacillus* and *Lactococcus* (65%–90%), with yeasts comprising the remainder (Simova et al., 2002; Rosa et al., 2017). Previously, homofermentative lactococci reportedly predominated (Koroleva, 1988a), but now *Lactobacillus* was confirmed to be the most numerous genus (at >92% of the bacterial content) (Marsh et al., 2013; Dallas et al., 2016; Walsh et al., 2016; Rosa et al., 2017). *Lb. kefiri*, *Lb. kefiranofaciens*, *Lb. paracasei* subsp. *paracasei*, *Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, and *Lb. plantarum* are most widely reported to predominate, while *Leuconostoc and Acetobacter* are also integral components of kefir grains (Table 12.1) (Anon., 2003; Sarkar, 2007; Chen et al., 2008; Da Cruz Pedrozo Miguel et al., 2010; Leite et al., 2012; Yang et al., 2014; Garofalo et al., 2015; Gao and Li, 2016; Walsh et al., 2016; Dertli and Çon, 2017; Rosa et al., 2017). However, there is considerable variation in terms of the organism(s) reported to predominate in the microbiota; the dominant organism(s) is variously identified as *Lb. kefiri* (Delfrederico et al., 2006; Da Cruz Pedrozo Miguel et al., 2010; Carasi et al., 2015; Toscano et al., 2017), or *Lb. kefiranofaciens* (Garofalo et al., 2015; Zanirati et al., 2015), or *Lb. kefiri* and *Lb. kefiranofaciens* (Leite et al., 2012; Likotrafiti et al., 2015), *Bacillus* spp. (Luang-In and Deeseenthum, 2016), *Leuconostoc mesenteroides* (with neither *Lb. kefiri*, nor *Lb. kefiranofaciens* present) (Leite et al., 2015), or *Lb. helveticus*, *Lb. kefiranofaciens* subsp. *kefiranofaciens*, *Lb. kefiranofaciens* subsp. *kefirgranum*, and other LAB (with *Lb. kefiri* not present) (Taş et al., 2012) or *Pseudomonas* sp. (with *L. mesenteroides*, *Lb. kefiranofaciens*, *Lb. kefiri* and other LAB) (Jianzhong et al., 2009).

The yeast population also displays considerable diversity, with both culture-dependent and culture-independent methods being utilized for isolation and identification. The most abundant yeasts in kefir (both grains and beverage) are *S. cerevisiae* (Leite et al., 2012; Kalamaki and Angelidis, 2017; Rosa et al., 2017), *Kluyveromyces marxianus* subsp. *marxianus* (previously *Saccharomyces kefir*), *Kazachstania unispora*, *Y. lipolytica* (Kalamaki and Angelidis, 2017; Rosa et al., 2017), *Saccharomyces unisporus*, *Candida kefir* (or *C. kefyr*), *Pichia fermentans*, *Torulaspora delbrueckii*, *Kazachstania aerobia*, and *Lachancea meyersii* (Rosa et al., 2017). A more comprehensive list of organisms isolated from kefir is supplied in Table 12.1.

As mentioned, it is widely acknowledged that the microbial population of different batches of kefir grains vary considerably due to the complex microbial association and the various factors that affects it (origin of grains, cultivation and storage practices, *inter alia*). At present, this also makes it well-nigh impossible to obtain a completely defined and constant kefir culture as a mother- or pure culture for industrial-scale operations (Drewek and Czarnocka-Roczniakowa, 1986; Gao and Li, 2016), but the application of emerging methodologies

Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage

Species	References
Lactobacilli	
Lactobacillus acidophilus (P)[a]	Taş et al. (2012), Nielsen et al. (2014), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017), Macori and Cotter (2018)
Lactobacillus amylovorus (P)	Bourrie et al. (2016), Gao and Li (2016)
Lactobacillus apis	Dertli and Çon (2017)
Lactobacillus brevis	Witthuhn et al. (2005), Rattray and O'Connell (2011), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus buchneri	Kesmen and Kacmaz (2011), Garofalo et al. (2015), Gao and Li (2016), Bourrie et al. (2016)
Lactobacillus bulgaricus	Frengova et al. (2002), Arslan (2015), Rosa et al. (2017), Macori and Cotter (2018)
Lactobacillus casei (P)	Bosch et al. (2006), Kesmen and Kacmaz (2011), Zanirati et al. (2015), Bourrie et al. (2016), De Paiva et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus crispatus (P)	Taş et al. (2012), Arslan (2015), Garofalo et al. (2015), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus curvatus (also water kefir)[b]	Witthuhn et al. (2004), Alzate et al. (2016)
Lactobacillus delbrueckii subsp. *bulgaricus* (P)	Frengova et al. (2002), Beshkova et al. (2003), Rattray and O'Connell (2011), Nielsen et al. (2014)
Lactobacillus delbrueckii subsp. *delbrueckii* (P)	Witthuhn et al. (2004), Nielsen et al. (2014)
Lactobacillus delbrueckii subsp. *lactis* (P)	Nielsen et al. (2014)
Lactobacillus diolivorans (also water kefir)	Kesmen and Kacmaz (2011), Zanirati et al. (2015), Bourrie et al. (2016), De Paiva et al. (2016)
Lactobacillus fermentum (also water kefir) (P)	Witthuhn et al. (2004, 2005), Rattray and O'Connell (2011), Nielsen et al. (2014), Bourrie et al. (2016), Randazzo et al. (2016), Rosa et al. (2017)
Lactobacillus fructivorans	Rattray and O'Connell (2011), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus gallinarum (P)	Nielsen et al. (2014), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus gasseri (P)	Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus gravieae	Bourrie et al. (2016)
Lactobacillus helveticus (P)	Frengova et al. (2002), Beshkova et al. (2003), Rattray and O'Connell (2011), Taş et al. (2012), Bourrie et al. (2016), Gao and Li (2016), Walsh et al. (2016), Rosa et al. (2017)
Lactobacillus hilgardii (also water kefir)[c]	Gulitz et al. (2011), Rattray and O'Connell (2011), Zanirati et al. (2015), Gao and Li (2016), Rosa et al. (2017)

Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	**References**
Lactobacillus hordei (also water kefir)	Gulitz et al. (2011), Hsieh et al. (2012), Gao and Li (2016)
Lactobacillus intestinalis	Garofalo et al. (2015), Bourrie et al. (2016)
Lactobacillus jensenii	Gao and Li (2016)
Lactobacillus johnsonii (P)	Nielsen et al. (2014), Bourrie et al. (2016)
Lactobacillus kalixensis	Bourrie et al. (2016)
Lactobacillus kefiri (also water kefir) (P)	Anon (2003), Bosch et al. (2006), Chen et al. (2008), Jianzhong et al. (2009), Kesmen and Kacmaz (2011), Rattray and O'Connell (2011), Garofalo et al. (2015), Prado et al. (2015), Zanirati et al. (2015), Bourrie et al. (2016), De Paiva et al. (2016), Gao and Li (2016), Walsh et al. (2016), Dertli and Çon (2017), Rosa et al. (2017), Taheur et al. (2017), Toscano et al. (2017)
Lactobacillus kefiranofaciens (POS)[d]	Chen et al. (2008), Kesmen and Kacmaz (2011), Rattray and O'Connell (2011), Prado et al. (2015), Zanirati et al. (2015), De Paiva et al. (2016), Gao and Li (2016), Lin et al. (2016), Walsh et al. (2016), Dertli and Çon (2017), Rosa et al. (2017)
Lactobacillus kefiranofaciens subsp. *kefiranofaciens*	Magalhães et al. (2010), Taş et al. (2012)
Lactobacillus kefiranofaciens subsp. *kefirgranum*	Bosch et al. (2006), Magalhães et al. (2010), Taş et al. (2012)
Lactobacillus kefirgranum	Bosch et al. (2006), Rattray and O'Connell (2011), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus mali (also water kefir)	Hsieh et al. (2012), De Paiva et al. (2016), Lin et al. (2016)
Lactobacillus mesenteroides	Rosa et al. (2017)
Lactobacillus nagelii (water kefir only)	Gulitz et al. (2011), Gao and Li (2016)
Lactobacillus otakiensis	Kesmen and Kacmaz (2011), Garofalo et al. (2015), Bourrie et al. (2016), Gao and Li (2016)
Lactobacillus parabuchneri	Bourrie et al. (2016), Gao and Li (2016)
Lactobacillus paracasei (P)	Da Cruz Pedrozo Miguel et al. (2010), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017), Bengoa et al. (2018)
Lactobacillus parafarraginis (also water kefir)	Zanirati et al. (2015), Bourrie et al. (2016), De Paiva et al. (2016)
Lactobacillus parakefir (also known as *Lb. parakefiri*)	Garrote et al. (2001), Rattray and O'Connell (2011), Hamet et al. (2015), Prado et al. (2015), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus plantarum (P)	Garrote et al. (2001), Bosch et al. (2006), Rattray and O'Connell (2011), Nielsen et al. (2014), Montijo-Prieto et al. (2015), Bourrie et al. (2016), Gao and Li (2016)

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Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	References
Lactobacillus pentosus	Bourrie et al. (2016)
Lactobacillus perolens (water kefir only)	Zanirati et al. (2015), De Paiva et al. (2016)
Lactobacillus reuteri (P)	Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus rhamnosus (P)	Rattray and O'Connell (2011), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactobacillus rossiae	Bourrie et al. (2016)
Lactobacillus sakei (P)	Nielsen et al. (2014), Bourrie et al. (2016)
Lactobacillus salivarius	Bourrie et al. (2016)
Lactobacillus sanfranciscensis	Walsh et al. (2016)
Lactobacillus satsumensis (POS)	Da Cruz Pedrozo Miguel et al. (2010), Bourrie et al. (2016), De Paiva et al. (2016), Fiorda et al. (2016), Gao and Li (2016)
Lactobacillus sunkii	Kesmen and Kacmaz (2011), Garofalo et al. (2015), Bourrie et al. (2016), Gao and Li (2016)
Lactobacillus ultunensis	Dertli and Çon (2017)
Lactobacillus uvarum	Da Cruz Pedrozo Miguel et al. (2010), Kesmen and Kacmaz (2011), Prado et al. (2015)
Lactobacillus viridescens	Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Lactococci	
Lactococcus filant	Angulo et al. (1993)
Lactococcus garvieae	Bourrie et al. (2016)
Lactococcus lactis subsp. *cremoris*	Yüksedağ et al. (2004), Rattray and O'Connell (2011), Bourrie et al. (2016), Rosa et al. (2017)
Lactococcus lactis subsp. *lactis*	Witthuhn et al. (2004), Yüksedağ et al. (2004), Witthuhn et al. (2005), Rattray and O'Connell (2011), Bourrie et al. (2016), Rosa et al. (2017)
Lactococcus lactis subsp. *lactis* biovar. diacetylactis	Garrote et al. (2001), Bourrie et al. (2016), Rosa et al. (2017)
Lactococcus lactis (also water kefir)	Frengova et al. (2002), Beshkova et al. (2003), Yüksedağ et al. (2004), Chen et al. (2008), Hsieh et al. (2012), Garofalo et al. (2015), Gao and Li (2016), Randazzo et al. (2016)
Lactococcus raffinolactis	Kesmen and Kacmaz (2011), Porcellato et al. (2015)
Streptococci	
Streptococcus cremoris	Yüksedağ et al. (2004), Rosa et al. (2017)
Streptococcus durans	Yüksedağ et al. (2004), Bourrie et al. (2016), Rosa et al. (2017)
Streptococcus faecalis	Rosa et al. (2017)
Streptococcus thermophilus	Frengova et al. (2002), Beshkova et al. (2003), Yüksedağ et al. (2004), Kesmen and Kacmaz (2011), Rattray and O'Connell (2011), Taş et al. (2012), Garofalo et al. (2015), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017), Macori and Cotter (2018)

Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	References
Leuconostocs	
Leuconostoc camosum	Walsh et al. (2016)
Leuconostoc citreum (also water kefir)	Gulitz et al. (2011), Walsh et al. (2016)
Leuconostoc gelidum	Walsh et al. (2016)
Leuconostoc kimchii	Walsh et al. (2016)
Leuconostoc lactis	Witthuhn et al. (2004), Rattray and O'Connell (2011), Gao and Li (2016)
Leuconostoc mesenteroides subsp. *cremoris*	Witthuhn et al. (2005), Rattray and O'Connell (2011), Bourrie et al. (2016)
Leuconostoc mesenteroides (also water kefir)	Garrote et al. (2001), Jianzhong et al. (2009), Gulitz et al. (2011), Kesmen and Kacmaz (2011), Rattray and O'Connell (2011), Hsieh et al. (2012), Bourrie et al. (2016), Gao and Li (2016), Randazzo et al. (2016), Walsh et al. (2016), Rosa et al. (2017)
Leuconostoc pseudomesenteroides	Kesmen and Kacmaz (2011), Bourrie et al. (2016)
Oenococcus oeni	Zanirati et al. (2015), Bourrie et al. (2016)
Acetic acid bacteria	
Acetobacter aceti	Rattray and O'Connell (2011), Rosa et al. (2017)
Acetobacter fabarum (also water kefir)	Gulitz et al. (2011), Garofalo et al. (2015), Gao and Li (2016)
Acetobacter lovaniensis	Garofalo et al. (2015), Prado et al. (2015), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Acetobacter orientalis (also water kefir)	Gulitz et al. (2011), Garofalo et al. (2015), Prado et al. (2015), Bourrie et al. (2016), Gao and Li (2016)
Acetobacter pasteurianus	Gao and Li (2016), Walsh et al. (2016)
Acetobacter rasens	Rattray and O'Connell (2011)
Acetobacter sicerae	Bourrie et al. (2016)
Acetobacter syzygii	Da Cruz Pedrozo Miguel et al. (2010), Kesmen and Kacmaz (2011), Gao and Li (2016), Rosa et al. (2017), Taheur et al. (2017)
Gluconoacetobacter diazotrophicus	Walsh et al. (2016)
Gluconobacter frateuri	Prado et al. (2015)
Gluconobacter japonicus	Da Cruz Pedrozo Miguel et al. (2010), Kesmen and Kacmaz (2011), Prado et al. (2015)
Gluconobacter oxydans	Walsh et al. (2016)
Other bacteria	
Acinetobacter calcoaceticus	Dertli and Çon (2017)
Acinetobacter rhizosphaerae	Dertli and Çon (2017)
Bacillus spp.	Garofalo et al. (2015), Fiorda et al. (2016), Luang-In and Deeseenthum (2016), Rosa et al. (2017)
Bacillus amyloliquefaciens	Luang-In and Deeseenthum (2016)

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Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	References
Bacillus megaterium (water kefir only)	Fiorda et al. (2016)
Bacillus siamensis	Luang-In and Deeseenthum (2016)
Bacillus tequilensis	Luang-In and Deeseenthum (2016)
Bacillus methylotrophicus	Luang-In and Deeseenthum (2016)
Bifidobacterium bifidum (P)	Taş et al. (2012), Rosa et al. (2017), Macori and Cotter (2018)
Bifidobacterium breve (P)	Dobson et al. (2011), Bourrie et al. (2016)
Bifidobacterium choerinum	Dobson et al. (2011), Bourrie et al. (2016)
Bifidobacterium longum (P)	Dobson et al. (2011), Bourrie et al. (2016)
Bifidobacterium pseudolongum	Dobson et al. (2011), Bourrie et al. (2016)
Bifidobacterium psychraerophilum	Hsieh et al. (2012)
Enterobacter amnigenus	Dertli and Çon (2017)
Enterobacter hormaechei	Dertli and Çon (2017)
Enterobacter soli	Dertli and Çon (2017)
Enterococcus durans	Sarkar (2007), Rattray and O'Connell (2011), Prado et al. (2015), Rosa et al. (2017)
Enterococcus faecalis	Sarkar (2007), Hsieh et al. (2012), Prado et al. (2015)
Enterococcus lactis	Dertli and Çon (2017)
Escherichia coli	Chen et al. (2008), Prado et al. (2015), Rosa et al. (2017)
Lysinibacillus sphaericus	Fiorda et al. (2016)
Pediococcus acidilactici	Arslan (2015), Rosa et al. (2017)
Pediococcus claussenii	Bourrie et al. (2016)
Pediococcus damnosus	Bourrie et al. (2016)
Pediococcus dextrinicus	Arslan (2015), Rosa et al. (2017)
Pediococcus halophilus	Bourrie et al. (2016)
Pediococcus lolii	Bourrie et al. (2016)
Pediococcus pentosaceus	Arslan (2015), Bourrie et al. (2016), Rosa et al. (2017)
Propionibacterium acnes	Dertli and Çon (2017)
Pseudomonas spp.	Chen et al. (2008)
Pseudomonas aeruginosa	Dertli and Çon (2017)
Pseudomonas azotoformans	Dertli and Çon (2017)
Pseudomonas otitidis	Dertli and Çon (2017)
Yeasts	
Brettanomyces anomalus	Rosa et al. (2017)
Candida albicans	Gao and Li (2016), Rosa et al. (2017)
Candida famata (also water kefir)	Alzate et al. (2016), Gientka et al. (2016)
Candida friedricchii	Rattray and O'Connell (2011), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)

Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	References
Candida guilliermondii	Gientka et al. (2016)
Candida holmii	Witthuhn et al. (2004), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Candida inconspicua	Alzate et al. (2016), Bourrie et al. (2016), Kalamaki and Angelidis (2017), Rosa et al. (2017)
Candida kefir (also known as *C. kefyr*)	Witthuhn et al. (2004), Nielsen et al. (2014), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Candida krusei (also water kefir)	Arslan (2015), Prado et al. (2015), Alzate et al. (2016), Rosa et al. (2017)
Candida lambica	Gao and Li (2016), Rosa et al. (2017)
Candida lipolytica	Witthuhn et al. (2004), Bourrie et al. (2016), Rosa et al. (2017)
Candida magnoliae (water kefir)	Alzate et al. (2016)
Candida maris	Rattray and O'Connell (2011), Bourrie et al. (2016), Rosa et al. (2017)
Candida parapsilosis	Dertli and Çon (2017)
Candida pseudotropicalis	Rosa et al. (2017)
Candida sake	Bourrie et al. (2016), Kalamaki and Angelidis (2017)
Candida sphaerica	Alzate et al. (2016)
Candida tannotelerans	Rosa et al. (2017)
Candida tenuis	Rosa et al. (2017)
Candida valida	Rosa et al. (2017)
Candida zeylanoides	Dertli and Çon (2017), Kalamaki and Angelidis (2017)
Cryptococcus humicolus	Witthuhn et al. (2005), Bourrie et al. (2016), Rosa et al. (2017)
Cryptococcus victoriae	Dertli and Çon (2017)
Davidiella tassiana	Marsh et al. (2013), Bourrie et al. (2016),
Dekkera anomala	Rattray and O'Connell (2011), Marsh et al. (2013), Garofalo et al. (2015), Prado et al. (2015), Bourrie et al. (2016)
Dekkera bruxellensis	Hsieh et al. (2012), Marsh et al. (2013), Bourrie et al. (2016)
Debaryomyces hansenii	Loretan et al. (2003), Rattray and O'Connell (2011), Kalamaki and Angelidis (2017), Rosa et al. (2017)
Debaryomyces occidentalis	Rattray and O'Connell (2011), Rosa et al. (2017)
Dipodascus geotrichum	Dertli and Çon (2017)
Eurotium amstelodami	Marsh et al. (2013), Bourrie et al. (2016)
Galactomyces geotrichum	Kalamaki and Angelidis (2017)
Geotrichium candidum	Arslan (2015), Bourrie et al. (2016)
Hanseniaspora guilliermondii	Garofalo et al. (2015)
Hanseniaspora uvarum	Fiorda et al. (2016)
Hanseniaspora valbyensis	Gulitz et al. (2011), Gao and Li (2016)
Issatchenkia occidentalis	Arslan (2015), Rosa et al. (2017)

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Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	References
Issatchenkia orientalis	Kalamaki and Angelidis (2017), Bourrie et al. (2016), Fiorda et al. (2016), Dertli and Çon (2017)
Kazachstania aerobia	Garofalo et al. (2015), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Kazachstania barnettii (aka *Saccharomyces barnetti*)	Marsh et al. (2013), Bourrie et al. (2016), Kalamaki and Angelidis (2017)
Kazachstania exigua	Jianzhong et al. (2009), Garofalo et al. (2015), Bourrie et al. (2016)
Kazachstania khefir	Prado et al. (2015)
Kazachstania servazzii	Garofalo et al. (2015), Bourrie et al. (2016), Gao and Li (2016), Taheur et al. (2017)
Kazachstania solicola	Garofalo et al. (2015), Bourrie et al. (2016), Gao and Li (2016)
Kazachstania turicensis	Garofalo et al. (2015)
Kazachstania unispora (aka *Saccharomyces unispora*)	Magalhães et al. (2010), Marsh et al. (2013), Gao and Li (2016), Garofalo et al. (2015), Dertli and Çon (2017), Kalamaki and Angelidis (2017)
Kluyveromyces dobzhanskii	Gao and Li (2016)
Kluyveromyces lactis	Loretan et al. (2003), Sarkar (2007), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Kluyveromyces marxianus	Magalhães et al. (2010), Taş et al. (2012), Marsh et al. (2013), Bourrie et al. (2016), Kalamaki and Angelidis (2017), Rosa et al. (2017)
Lachancea fermentati (water kefir only)	Gulitz et al. (2011), Fiorda et al. (2016), Gao and Li (2016)
Lachancea meyersii	Nielsen et al. (2014), Prado et al. (2015), Rosa et al. (2017)
Naumovozyma spp.	Prado et al. (2015), Bourrie et al. (2016)
Naumovozyma castelli (aka *Saccharomyces castelli*)	Marsh et al. (2013), Bourrie et al. (2016)
Pichia caribbica	Gao and Li (2016)
Pichia cecembensis	Gao and Li (2016)
Pichia fermentans	Rattray and O'Connell (2011), Hsieh et al. (2012), Marsh et al. (2013), Bourrie et al. (2016), Gao and Li (2016), Kalamaki and Angelidis (2017), Rosa et al. (2017)
Pichia kudriavzevii (aka *Issatchenkia orientalis, Candida crusei*)	Marsh et al. (2013), Arslan (2015), Prado et al. (2015), Fiorda et al. (2016)
Pichia membranifaciens	Fiorda et al. (2016), Kalamaki and Angelidis (2017)
Rhodutorula dairenensis	Dertli and Çon (2017)
Rhodutorula mucilaginosa	Dertli and Çon (2017)
Saccharomyces cariocanus	Garofalo et al. (2015), Bourrie et al. (2016)
Saccharomyces carlsbergensis	Brialy et al. (1995)

Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	References
Saccharomyces cerevisiae	Anon (2003), Beshkova et al. (2003), Magalhães et al. (2010), Marsh et al. (2013), Bourrie et al. (2016), Fiorda et al. (2016), Gao and Li (2016), Walsh et al. (2016), Dertli and Çon (2017), Kalamaki and Angelidis (2017), Rosa et al. (2017)
Saccharomyces delbrueckii	Anon (2003), Rattray and O'Connell (2011), Rosa et al. (2017)
Saccharomyces exiguus	Anon (2003), Witthuhn et al. (2004), Gao and Li (2016), Rosa et al. (2017)
Saccharomyces fragilis	Anon (2003), Arslan (2015), Rosa et al. (2017)
Saccharomyces humaticus	Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Saccharomyces lactis	Arslan (2015), Rosa et al. (2017)
Saccharomyces lipolytica	Golowczyc et al. (2009), Arslan (2015), Rosa et al. (2017)
Saccharomyces pastorianus	Rattray and O'Connell (2011)
Saccharomyces rouxii	Loretan et al. (2003)
Saccharomyces turicensis	Rattray and O'Connell (2011), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Saccharomyces unisporus	Loretan et al. (2003), Rattray and O'Connell (2011), Bourrie et al. (2016), Gao and Li (2016)
Torula kefir	Macori and Cotter (2018)
Torulopsis holmii	Rosa et al. (2017)
Torulaspora delbrueckii	Loretan et al. (2003), Rattray and O'Connell (2011), Nielsen et al. (2014), Bourrie et al. (2016), Gao and Li (2016), Rosa et al. (2017)
Wallemia sebi	Marsh et al. (2013), Bourrie et al. (2016)
Weissella sp.	Prado et al. (2015), Rosa et al. (2017)
Wickerhamomyces anomalus	Kalamaki and Angelidis (2017)
Yarrowia lipolytica	Rattray and O'Connell (2011), Nielsen et al. (2014), Bourrie et al. (2016), Dertli and Çon (2017), Kalamaki and Angelidis (2017), Rosa et al. (2017)
Zygosaccharomyces spp.	Witthuhn et al. (2005), Arslan (2015), Bourrie et al. (2016), Rosa et al. (2017)
Zygosaccharomyces fermentati	Gulitz et al. (2011), Hsieh et al. (2012), Fiorda et al. (2016), Gao and Li (2016)
Zygosaccharomyces lentus	Marsh et al. (2013), Bourrie et al. (2016)
Zygosaccharomyces rouxii	Sarkar (2007), Arslan (2015)
Zygotorulaspora florentina (water kefir only)	Gulitz et al. (2011)
Molds	
Alternaria spp.	Dertli and Çon (2017)
Aspergillus spp.	Dertli and Çon (2017)

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Table 12.1 Microorganisms Reported in Kefir Grains and the Kefir Beverage—cont'd

Species	References
Aspergillus amstelodami	Dertli and Çon (2017)
Mucor circinelloides	Dertli and Çon (2017)
Penicillium sp. *Vega* 347	Marsh et al. (2013), Bourrie et al. (2016)

[a] P signifies organisms widely reported and/or used in commercial applications as probiotic strains (Vasiljevic and Shah, 2008; Illanes and Guerrero, 2016).
[b] Reported in water kefir grains only.
[c] Reported in both milk and water kefir grains.
[d] POS signifies organisms identified to have probiotic potential (Chen et al., 2017b).

may change this in the future, as is discussed later. Scanning electron micrographs (SEMs) of kefir grains also confirm this diversity (Marshall, 1984a; Rea et al., 1996; Garofalo et al., 2015). SEM clearly indicate that, both between different grain cultures, as well as within the grains of one culture, the distribution of the constituent microorganisms vary among different areas of the grain: typically, lactose-fermenting yeasts and lactobacilli (predominantly short bacilli) occupy the outside regions (Güzel-Seydim et al., 2005; Garofalo et al., 2015; Kesenkas et al., 2017), while a variety of lactobacilli (short, long, and curved) predominate in the central regions (Marshall et al., 1984; Rea et al., 1996; Güzel-Seydim et al., 2005; Gao and Li, 2016). In the intermediate region, yeasts and bacteria are present in similar numbers (Kesenkas et al., 2017). Some authors observed cocci (Rea et al., 1996), reportedly associated with the yeasts (Rattray and O'Connell, 2011), but most did not. However, their absence is more than likely due to poor attachment by lactococci, making them vulnerable to the relatively harsh sample preparation required for SEM (Güzel-Seydim et al., 2005; Gao and Li, 2016). Culture-independent techniques (16S rDNA gene sequence analysis) also confirmed that the interior regions of the grains showed greater microbial diversity than the exterior regions (Dobson et al., 2011), with *Lb. kefiranofaciens* reported to appear throughout the grains, but dominating the central region, while *Lb. kefiri* dominates the outside region (Otles and Cagindi, 2003; Kesenkas et al., 2017). Even the appearance of the embedding matrix varies from resembling cobwebs, or having a honey-comb structure (Marshall, 1984a; Rea et al., 1996), to displaying a fibrillar appearance (Güzel-Seydim et al., 2005; Gao and Li, 2016), as seen in SEM.

However, the fact that kefir grains survive under conditions that do not preclude other microorganisms, suggests that at least part of the microbial population is specific and constant (Marshall et al., 1984). This suggestion is confirmed by the observation that, when different grain samples were cultivated under the same conditions, their microbial populations became very similar (Koroleva, 1988a). Moreover, the observed 78%–94% similarity among bacterial spp. and 80%–92% similarity among yeast spp. among three different Tibetan kefir cultures (Jianzhong et al., 2009), further supports this suggestion.

On the other hand, the significant variation in the composition of the microbiota reported in various studies may be attributed to the fact that the methods used for identification were inadequate to detect and describe the diversity. While these traditional (culture-dependent) techniques are still believed to have a role to play in the detection and enumeration of some organisms (Chen et al., 2008; Kesmen and Kacmaz, 2011), they have to be augmented by culture-independent methods in the interest of accuracy. Moreover, the application of methods capable of identifying and quantifying all of the microorganisms in the grains has gained more importance in view of the resurgent interest in kefir as a probiotic product (Rosa et al., 2017), while identification of the probiotic organism by genus, species, and strain is vital in order to make probiotic claims (Mizock, 2015). Since traditional culture-dependent techniques are often inadequately selective, thus providing only partial identification of the complete microbiota, culture-independent techniques have been employed since the end of the 1990s and are continuously being refined (Magalhães et al., 2010; Dobson et al., 2011; Marsh et al., 2013). Application of these techniques in recent years allowed the identification of a considerable number of organisms that were previously not known to be present in kefir (Marsh et al., 2013; Rosa et al., 2017), explaining the substantial difference between the 30 species reported by Nielsen et al. (2014) and the 300 plus species reported 3 years later by Rosa et al. (2017). Moreover, while the organisms reported in Table 12.1 have all been isolated from kefir, some may be contaminants, emphasizing the need for further refinement in characterization methodologies, as well as comprehensive studies around the world in order to clearly define the microbiota in kefir grains from different geographical locations (Rattray and O’Connell, 2011).

Nowadays, culture-independent, molecular profiling techniques are used widely toward elucidating aspects of the microbiota (Leite et al., 2015; Mizock, 2015; Dallas et al., 2016; Yazdi et al., 2017). Amplicon-based sequencing is widely applied when the objective is taxonomy and enumeration of the diverse taxa (Bokulich et al., 2016; Ferrocino and Cocolin, 2017). Most of these massively parallel, high-throughput sequencing (HTS) techniques start with PCR, that is, involve the amplification of DNA extracted from the food matrix, followed by further analyses to identify the differences in the amplified

DNA sequences. Pure isolates are used and genes selected for amplification are typically those encoding for ribosomal RNA (rRNA), with regions of the 16S rRNA coding gene used for bacteria and regions of the 26S rRNA coding gene used for yeasts (Jianzhong et al., 2009; Cocolin et al., 2013; Bokulich et al., 2016; Ferrocino and Cocolin, 2017). Selecting rRNA as a target in favor of DNA ensures that the analysis enumerates live and active microorganisms, but since RNA may lead to quantitative over- or underestimation due to the wide variation in operon copy number across taxa, many studies of dairy and other food ecosystems used the 16S DNA gene (microbiome) and the 26S DNA or internal transcribed space (ITS) gene (mycobiome) as the targets. Delfrederico et al. (2006) successfully sequenced the ITS gene (the spacer region between the 16S and 23S rRNA genes), enabling identification to species level. However, it appears that targeting the D1/D2 region of the 26S rRNA gene, using NL1 primers ensures the most reliable identification of fungal diversity (Ferrocino and Cocolin, 2017). Real time, or quantitative PCR (QPCR) can also be used to estimate the total bacterial and yeast concentration (Porcellato et al., 2015; Kim et al., 2016b; Walsh et al., 2016; Dallas et al., 2016; Kim et al., 2018).

The application of a shotgun approach, that is, where the entire genome contents of the matrix or ecosystem are sequenced is known as metagenome sequencing and obviates the need for pure cultures (Ferrocino and Cocolin, 2017). This can result in the identification of individual genomes or genome fragments of taxonomic interest as well as of functional genes (Bokulich et al., 2016; Ferrocino and Cocolin, 2017). These techniques are facilitated by several HTS or next-generation sequencing (NGS) data analysis platforms and chemistries (Bokulich et al., 2016; Tessler et al., 2017), for example pyrosequencing, Illumina 454, and nanopore sequencing, as opposed to DGGE (a low-throughput sequencing technique) (Cocolin et al., 2013; Bokulich et al., 2016; Ferrocino and Cocolin, 2017). Moreover, advances in next generation "OMICS" methods enable the discovery of more detail about the microbiota and the process (Heinl and Grabherr, 2017). For example, a comprehensive study of a multispecies and multiorganism food ecosystem, such as kefir, as well as how they interact with the environment and with each other (i.e., the process) could entail: amplicon sequencing (accurate identification of microbial composition); metagenomics (gene content), metatranscriptomics (gene function); metaproteomics (functional activity); and meta-metabolomics (metabolism/metabolites). In addition, advances in bioinformatics and biostatistics, as well as the availability of online data analysis platforms, progressively facilitates the complete description of even complex ecosystems, the interactions and changes that take place during fermentation and the influence of environmental factors (Kergourlay et al., 2015; Ferrocino and Cocolin, 2017). Hence, it is reasonable to project that holistic integration of these meta-omic tools will lead to complete functional characterization and more efficient production of fermented foods (Chen et al., 2017a), including kefir.

Studies where these techniques were applied to kefir are numerous, but a few examples follow. Amplification of fungal ITS rDNA fragments, followed by PCR-DGGE was used to identify the yeasts in kefir (Magalhães et al., 2010; Marsh et al., 2013). However, exploiting its greater sensitivity toward accurate identification (Tessler et al., 2017), HTS has been used instead of DGGE to elaborate the microbial diversity in Brazilian kefir grains (Cocolin et al., 2013), while Garofalo et al. (2017) found length-heterogeneity (LH)-PCR more sensitive than PCR-DGGE in terms of detecting LAB in foods (including kefir). Using a HT parallel sequencing approach as well as 16S compositional sequencing, Dobson et al. (2011) were able to quantify the various genera more accurately, reporting greater than a 1000-fold difference in presumptive *Lactococcus* relative to *Lactobacillus* population and were able to detect *Bidifobacterium* spp. in kefir for the first time, while culture-dependent techniques were unable to detect these organisms. Repetitive extragenic palindromic sequence-based PCR (rep-PCR) fingerprinting allowed primary differentiation and grouping, after which the results were validated using 16S rDNA gene sequence analysis, also leading to identification of hitherto undetected organisms in kefir grains and beverages (Kesmen and Kacmaz, 2011; Leite et al., 2015). Dallas et al. (2016) used shotgun metagenomic sequencing to identify LAB in kefir, coupled with peptidomics to describe their proteolytic activity. Walsh et al. (2016) combined different OMICS approaches to get a more holistic picture. These authors used two HTS techniques, namely amplicon sequencing (16S rRNA and ITS) as well as whole-metagenome shotgun sequencing to elucidate and enumerate bacteria and fungi in kefir. They augmented this with metabolomics and flavor analysis, with all analyses performed in a time-dependent fashion, enabling not only accurate identification of the microbiota constituents, but also the dynamic pattern of microbial succession, linked to the flavor compounds elaborated by specific organisms. In addition, genes encoding proteins important to probiotic activity were identified in *Lb. kefiranofaciens*, further illustrating the holistic nature of the information gleaned by applying the culture-independent techniques in question. In addition, the application of metabolomics, transcriptomics, and genetic analysis, as well as a genome-scale community metabolic prediction model, tested with experimental results (genomics), enabled insight into the naturally established, complex dynamic multi-metabolite cross-feeding between a typical kefir yeast and LAB (including *L. lactis*, isolated from kefir). Since an understanding of the regulatory processes, as well as the metabolic fluxes of several hundreds of metabolites was gained, the results paved the way for exploiting nutrient balancing and pathways to regulate species dynamics in yeast-LAB symbioses (Ponomarova et al., 2017). The results of Ponomarova et al. (2017) and Walsh et al. (2016) suggest a potential route toward formulating the ideal kefir culture and toward engineering predictable kefir fermentations in the future.

12.2.2 Formation of the Kefir Grains

Kefir grains do not form when combining pure cultures of the constituent organisms, but kefir-like grains were produced when imitating the traditional process of fermenting milk in bags made from animal skins (goat or sheep) (Otles and Cagindi, 2003; Güzel-Seydim et al., 2010; Nielsen et al., 2014; Bourrie et al., 2016). These grains were produced using an intricate process of inoculating sheep intestinal microorganisms into pasteurized cow's milk in a goatskin pouch. Incubation with agitation occurred for 48 h at 24–26°C, after which 75% of the coagulated milk was replaced with fresh milk. This process was repeated for 12 w, after which the water-insoluble gel-like layer that was removed from the inside of the pouch was propagated in milk, resulting in a structure that resembled kefir grains (Rattray and O’Connell, 2011; Nielsen et al., 2014; Kesenkas et al., 2017).

12.2.3 Propagation of the Kefir Starter

The kefir grains is also the starter or mother culture (Kroger, 1993) that is typically used repeatedly since it remains active indefinitely if maintained correctly (Shavit, 2008; Rattray and O’Connell, 2011). Hence, kefir grains could be considered a nature-made immobilized culture (Rimada and Abraham, 2006; Van Wyk et al., 2011; Rosa et al., 2017). CODEX STAN 243-2003 is the Codex Alimentarius Standard for fermented milks which defines the kefir starter as follows: “Starter culture prepared from kefir grains, *Lb. kefiri*, species of the genera *Leuconostoc*, *Lactococcus*, and *Acetobacter* growing in a strong specific relationship. Kefir grains constitute both lactose fermenting yeasts (*Kluyveromyces marxianus*) and nonlactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*)” (Anon., 2003). However, as discussed elsewhere and as depicted in Table 12.1, kefir grains comprise a considerably more complex microbiota than those mentioned in the Codex Standard. It needs to be noted that *Lactobacillus kefiri* is also known as *Lb. kefir* (Farnworth, 2005; Rimada and Abraham, 2006; Rattray and O’Connell, 2011; Carasi et al., 2015; Rosa et al., 2017), but since it is identified in the Codex Standard (Anon., 2003) as *Lb. kefiri*, the latter was used throughout this chapter.

New starters for kefir are customarily obtained and propagated by growing grains in skimmed milk and then subdividing the culture

(Koroleva, 1988a), since the biomass of kefir grains increases when cultivated in milk (Kroger, 1993; Garrote et al., 2001) and other substrates (Alzate et al., 2016). The increased biomass comprises both matrix and microbiota (Garrote et al., 2001; Gao and Li, 2016), with a 5%–11% increase in the mass of kefir grains per day during continuous cultivation in milk at 30°C possible (Libudzisz and Piatkiewicz, 1990; Güzel-Seydim et al., 2010; Rattray and O'Connell, 2011), but a more moderate 2% increase indicated as the norm (Rosa et al., 2017). Cultivation of kefir grains using synthetic media with a view to produce large quantities of biomass is feasible. Glucose:sucrose in a 1:3 ratio in whole (Harta et al., 2004) or in low-fat milk augmented with yeast extract and tryptose resulted in substantial biomass production, but the resultant kefir beverage lacked flavor due to an imbalance in the microbiota, notably the yeasts (Schoevers and Britz, 2003). This problem was resolved by Exarhopoulos et al. (2017) who propagated kefir grains in UHT skimmed milk and succeeded in producing 26.68 kg of grains from a starting mass of 80 g.

After propagation, the grains can be used directly for inoculation into a fresh batch of milk (Kroger, 1993; Rosa et al., 2017), but postpropagation grain storage is often the norm, hence the grains are subjected to storage at reduced temperature and/or drying prior to storage. However, storage affects the microbial composition and the viability of the grains. Grains stored at 18°C for 10 days under aerobic conditions, under a thin layer of milk, resulted in excessive yeast growth (Drewek and Czarnocka-Roczniakowa, 1986), while storage at 4°C is reported to be the least favorable, leading to loss of activity within 8–10 d (Garrote et al., 1997; Güzel-Seydim et al., 2010; Rosa et al., 2017). In contrast, storage in milk at −80°C best preserved the kefir grains, with storage at −20°C, that is, domestic freezer conditions (Güzel-Seydim et al., 2010), and even 10°C (domestic refrigerator conditions) produce grains of satisfactory quality. As mentioned, drying is also used as a preservation technique and facilitates commercial distribution (Koroleva, 1988a; Kroger, 1993; Rea et al., 1996; Rosa et al., 2017). Freeze-drying is widely acknowledged to ensure maximum survival rate of the organisms (Atalar and Dervisoglu, 2015), but drying at room temperature, or at 33°C, with or without vacuum (Papapostolou et al., 2008), and spray-drying (Atalar and Dervisoglu, 2015) also showed good retention of microbial activity upon reconstitution. For example, kefir grains dried for 36–48 h at room temperature retained activity upon reconstitution after being stored for 12–18 months (Koroleva, 1988a; Kroger, 1993; Rea et al., 1996; Rosa et al., 2017). After drying and/or storage, the grains can be reconstituted or reactivated by repeated incubations in fresh milk (Kosikowski, 1982; Rosa et al., 2017).

Although the best quality kefir, that is, a kefir beverage with the distinctive complex flavor and microbiota, is as yet only attainable when using grains (Arslan, 2015; Rosa et al., 2017), microorganisms are shed from the grains into the fermenting milk (fermentate). This fermentate is used to produce kefir on a commercial scale by inoculating milk with 2%–7% of this "bulk starter." This bulk starter is also known as the percolate (i.e., the liquid obtained when separating the grains). However, although this fermentate or percolate (in either fresh or freeze-dried form) when used as bulk starter achieves fermentation, the resultant beverage will not be the same as with kefir grains (Libudzisz and Piatkiewicz, 1990; Rea et al., 1996; Rattray and O'Connell, 2011). This is due to the fact that the microbial population of these alternative inocula is also not the same as that of the grains (Farnworth, 2005; Nielsen et al., 2014). In particular, lower concentrations of yeasts are observed in the beverage and the fermentate. Hence, due to the important role of yeasts in flavor and CO_2 production, the deleterious effect on beverage quality is to be anticipated (Duitschaever, 1989; Farnworth, 2005). Duitschaever (1989) overcame this problem by adding a yeast culture after the lactic starter, while Clementi et al. (1989) employed immobilized yeast cells with a grain-free starter.

An alternative approach is to obtain grains or at least a close simulation of their complex microbiota by starting with mixed pure or crude cultures of organisms present in the original grains. After numerous attempts to this end had failed (Liu and Moon, 1983; Koroleva, 1988a; Libudzisz and Piatkiewicz, 1990), Beshkova et al. (2002, 2003) achieved success using a starter culture comprising microorganisms isolated from kefir (*Streptococcus thermophilus*, *Lb. bulgaricus*, *Lb. helveticus*, *Lactococcus lactis*, and *S. cerevisiae*) and well-defined process conditions. The resultant kefir-like beverage had sensory qualities close to, and even exceeding, that of the traditional beverage, with the viscosity, in particular, similar to that of kefir produced with grains (Beshkova et al., 2002; Frengova et al., 2002). The sample most preferred by the sensory panel involved consecutive lactic acid and yeast fermentations, with the lactic acid fermentation at 28°C and the yeast fermentation in the bottle at 20°C (Beshkova et al., 2002). In fact, compared to the samples inoculated with kefir grains, the grain-free starter produced higher concentrations of volatiles, like diacetyl, acetaldehyde, acetone, and ethanol, resulting in a beverage with the unique aroma and flavor characteristic of a good quality kefir (Beshkova et al., 2003). The noteworthy successful attempts at using grain-free starters by these authors are also attributable to the inclusion of the specific *Lb. bulgaricus* strain in the starter. Demonstrating enhanced biosynthesis in the presence of the other LAB and the yeast, this strain produced kefiran of similar quality and in similar quantities compared to kefir produced using grains (Frengova et al., 2002). The aforementioned starters were in freeze-dried or lyophilized format which facilitates culture maintenance and handling, hence they are used commercially (Libudzisz and Piatkiewicz, 1990; Rattray and O'Connell, 2011) as direct vat inoculation (DVI) or direct vat set (DVS) cultures (Rattray and O'Connell, 2011; Prado et al., 2015). However, during the freeze-drying process, it is vital to use a cryoprotectant, such as whey, to ensure survival of the microbiota (Papavasiliou et al., 2008). Chen et al. (2009) attempted to produce defined cultures in a format that imitate kefir grains. They produced microspheres in which they immobilized selected kefir bacterial and yeast strains separately. The ratio of organisms mimicked the distribution found in kefir grains, enabling them to produce a kefir beverage, after which the microspheres were successfully reused during 28 successive fermentation cycles. Prado et al. (2015) reported that a balanced fermentation can be achieved, as well as the probiotic effect be enhanced, by using adjunct cultures (*Bifidobacterium* spp., *Lactobacillus* spp. and *Saccharomyces boulardii*, a probiotic yeast) with the grains or the DVI/DVS freeze-dried preparations. Moreover, by using a time-dependent approach to elucidate the whole genome of the microbiota in kefir grains and kefir, while applying metabolomics to monitor metabolites and metabolic fluxes, it is possible to accurately define the ideal ecosystem, hence enabling the formulation of superior multi-strain starter cultures capable of conducting a predictable fermentation, resulting in well-defined product quality parameters (Walsh et al., 2016). Moreover, DVI commercial culture preparations not only simplifies the production process, but another advantage is that the beverage produced in this way has superior shelf life to its grain-produced counterpart, namely up to 28 d, compared to 1.5–12 d (Koroleva, 1988b; Rattray and O'Connell, 2011). Hence, with these advances in mind, the possibility exists that standardized commercial cultures, either in freeze-dried or immobilized format, capable of producing a high-quality kefir beverage, with the requisite sensorial attributes and probiotic functionalities, are within reach.

12.3 The Manufacturing Process

12.3.1 The Substrates

Although cow's milk is the most common substrate, kefir is also produced from sheep's, goat's, buffalo's (Nielsen et al., 2014; Arslan, 2015; Addeo et al., 2007; Rosa et al., 2017), and camel's milk (Rosa et al., 2017) as well as from whey (Magalhães et al., 2010, 2011; Gao and Li, 2016). Nondairy sources include milk substitutes, namely soy (Baù et al., 2013, 2015), walnut, coconut, rice, and peanut milk (Bensmira and Jiang, 2012; Cui et al., 2013; Nielsen et al., 2014; John and Deeseenthum, 2015; Gao and Li, 2016), as well as a variety of sugary liquid substrates, including sugar cane, molasses, brown sugar, fruit and vegetable juice, beer wort, ginger beer-base, coconut water, cocoa pulp (Silva et al., 2009; Puerari et al., 2012; Baù et al., 2013; Nielsen et al., 2014; Baù et al., 2015; Alzate et al., 2016; Corona et al., 2016; Gao and Li, 2016; Randazzo et al., 2016; Zanirati et al., 2015; Fiorda et al., 2017), and honey (Fiorda et al., 2016). The milk substitute substrates may require supplementation with lactose, sucrose, or glucose (Baù et al., 2013; Nielsen et al., 2014; Baù et al., 2015), but postfermentation, all nondairy kefirs sustain the growth of the LAB and yeasts arising from the grain microbiota, as do their dairy counterparts (Silva et al., 2009; Baù et al., 2013; Corona et al., 2016; Fiorda et al., 2016; Gao and Li, 2016; Fiorda et al., 2017), confirming their suitability as probiotic beverages for consumers with dairy allergies and for vegans (Fiorda et al., 2016; Corona et al., 2016; Fiorda et al., 2016). Moreover, the grains used to inoculate the nondairy substrates are not the same as their dairy counterparts. Liu et al. (2002a) described soymilk kefir grains, while the sugary substrates are typically inoculated with water kefir grains (also known as sugar kefir or sugar water kefir grains) (Corona et al., 2016; De Paiva et al., 2016; Gao and Li, 2016). However, these fermentations also result in beverages with a complex flavor profile (Corona et al., 2016; Liu et al., 2002a; Randazzo et al., 2016), resulting in consumer acceptance, notably of apple-, grape- (Randazzo et al., 2016), carrot- (Corona et al., 2016), and cocoa pulp-based beverages, the latter of which had a relatively high ethanol content of 3.6% (v/v) (Puerari et al., 2012). Of special interest are the whey-fruit juice-based kefir beverages which not only showed a desirable sensory profile, but also sustained survival of live probiotic organisms in the fermented beverage. These beverages are also reminiscent of the commercially available fruit kefirs, that is, milk-based kefir flavored with fruit (Kroger, 1993; Farnworth, 2005; Sabokbar and Khodaiyan, 2015). Moreover, both the whey- and cocoa pulp-based beverages offer an attractive waste-beneficiation option (Puerari et al., 2012; Gao and Li, 2016).

12.3.2 The Process

The first kefir production occurred at domestic level and the practice of producing an artisanal home-fermented beverage from shared grains is still common practice around the world (Nielsen et al., 2014; Rosa et al., 2017). At the end of the fermentation, the grains are separated from the beverage and are used to produce a fresh batch of kefir beverage, obviating the necessity for back-slopping, a practice where a portion of the previous batch of fermented milk constitutes the inoculum, which is prone to failure due to contamination (Nielsen et al., 2014).

The best quality kefir is obtained when ensuring a balanced fermentation, achieving the ideal pattern of microbial succession (Walsh et al., 2016). To this end, Koroleva (1988b) advised the following regime as the ideal for the manufacture of kefir: inoculation with grains [2%–5% (w/v)], fermentation at 20–22°C for 10–12 h, agitation and removal of the grains, followed by slow cooling to, and then ripening at, 8–10°C over the next 10–12 h. However, this traditional process is not feasible at industrial scale. Firstly, the relative surface area of the grains to the volume of milk will make fermentation nonhomogeneous and grain retrieval onerous. Hence, the industrial process utilizing grains is fashioned upon the Russian or European method, which starts with a 2%–10% (w/v) grain inoculum into pasteurized milk, fermentation (20–25°C for 18–24 h), followed by grain separation via straining or filtration. The grains are retained for reuse while the filtrate is inoculated into fresh pasteurized milk at 1%–3% (v/v) to produce a batch culture after 24 h at 20°C. This batch culture is then inoculated into a fresh batch of pasteurized milk at 2%–7% (w/v), followed by further fermentation at 19–25°C for 12–18 h, after which the product is slow cooled and then held at 8–10°C for 8–12 h (Koroleva, 1988b; Mann, 1989; Rattray and O'Connell, 2011; Nielsen et al., 2014; Kesenkas et al., 2017). During this cooling stage, the second stage of the fermentation, comprising an alcoholic fermentation with concomitant CO_2 production and further flavor development, occurs (Rattray and O'Connell, 2011; Rosa et al., 2017). Reminiscent of the secondary fermentation during Champagne production, maximum effervescence of the commercial kefir beverage is achieved by executing the second stage in crown-capped bottles (Roginski, 1988). This second fermentation stage is also considered a maturation process since more flavor develops, while lactose levels decrease, making the product suitable for diabetics and lactose intolerant individuals (Rosa et al., 2017).

The second route of industrial scale kefir production involves DVI/DVS application of 2%–8% of a commercial culture preparation into the milk or other substrate, followed by incubation at 20–30°C for 10–16 h, until a pH of 4.5–4.6 is reached (Rattray and O'Connell, 2011; Prado et al., 2015; Kesenkas et al., 2017). All processes (traditional, Russian/European, or DVI) culminate in cold storage of the final product at 4–6°C (Rattray and O'Connell, 2011; Kesenkas et al., 2017). While unpasteurized milk can be used for household kefir production (John and Deeseenthum, 2015), the commercial processes involve pasteurization of milk. The milk is heated at 90–95°C for 5–15 min, followed by cooling to the inoculation temperature (18–30°C), with the higher temperature (30°C) applied in some processes where commercial cultures are used (Rattray and O'Connell, 2011; Kesenkas et al., 2017). Homogenization is also performed, either before (Koroleva, 1988b; Mann, 1989) or after pasteurization (Rattray and O'Connell, 2011), while incubation in the final package or in a bulk container produces either set kefir or stirred kefir, respectively (Rattray and O'Connell, 2011).

12.4 The Kefir Beverage

12.4.1 Characteristics of the Kefir Beverage

The characteristics of the beverage encompass the viscosity, the flavor, as well as the chemical and microbial composition. These are influenced by the type of milk, the composition and concentration of the inoculum (grains or cultures), as well as the manufacturing process, including the use or the absence of agitation during fermentation, as well as the temperature and rate of cooling and ripening postfermentation (Garrote et al., 1998; Farnworth, 2005; Arslan, 2015; Rattray and O'Connell, 2011; Rosa et al., 2017).

A good kefir beverage has a viscous, yet pourable consistency, is foamy like beer, with a unique flavor, distinct yeasty and pleasant acidic taste and effervescent mouth feel. As mentioned previously, the flavor derives from diacetyl and other volatile compounds, while lactic acid and CO_2 cause the acidic taste and effervescence, respectively (Marshall, 1984b; Farnworth, 2005; Arslan, 2015).

Although the flavor of kefir is exceptionally complex, the main flavor compound is diacetyl or 2,3-butanedione, the ketone with the pronounced trademark buttery aroma. Kefir contains 0.3–1.0 mg/L diacetyl, which is produced by *L. lactis* (Libudzisz and Piatkiewicz, 1990; Hugenholtz, 2008; Rattray and O'Connell, 2011) and/or *L. mesenteroides* (Rattray and O'Connell, 2011; Walsh et al., 2016), 0.88–4.40 μg/mL acetaldehyde, as well as 53.1–67.8 mg/L acetoin or 3-hydroxy-2-butanone (Libudzisz and Piatkiewicz, 1990; Otles and Cagindi, 2003; Aghlara et al., 2009) and 2.96–3.03 mg/L acetone (Aghlara et al., 2009). The complexity of the flavor is abundantly evident from the results of a recent study where an additional 36 volatile compounds were identified and described. Apart from the anticipated "buttery" note, the aroma descriptors include notes like "fruity," "floral," "malty," "apple," "grape," "sweet," "creamy," "caramellic," "spicy," "orange peel," "cognac," as well as "sweaty," "sulfurous," and even "goaty" (Walsh et al., 2016). ʟ(+) lactic acid (with minor levels of the ᴅ(−) form) is the major organic acid present, typically increasing from 0.1% to 1.0% (v/v) during the fermentation, concomitant with a 20%–30% (w/v) decrease in lactose (Otles and Cagindi, 2003; Farnworth, 2005; Arslan, 2015; Kesenkas et al., 2017; Rosa et al., 2017) and a final pH of 4.2–4.6 (Marshall, 1984a; Farnworth, 2005; Arslan, 2015). Formic and succinic acids are also present, while hippuric, pyruvic, acetic, propionic, and butyric acids are considered minor flavor compounds (Libudzisz and Piatkiewicz, 1990; Sarkar, 2007; Arslan, 2015;

Rosa et al., 2017). Moreover, Walsh et al. (2016) demonstrated a causal relationship between the changing relative abundance of members of the microbiota during the fermentation and the flavor compounds biosynthesized by these constituent organisms. As such, *Lactobacillus* spp. correlated with carboxylic acids, esters (fruity flavors), and ketones (cheesy flavors), *Leuconostoc* spp. with acetic acid (vinegary) and 2,3-butanedione (buttery), *Acetobacter pasteurianus* with acetic acid and *S. cerevisiae* with esters.

Kefir contains biogenic amines, such as putrescine, cadaverine, penicillamine, and histamine which are correlated with an undesirable bitter taste and could precipitate pseudoallergic reactions (Nuñez et al., 2016), cytotoxic effects, and other disorders (Ordóñez et al., 2016). However, the total bioactive amine content in kefir ranges from 2.4 to 35.2 mg/L (with putrescine and tyramine the most prevalent) (Ordóñez et al., 2016; Rosa et al., 2017), concentrations that are considerably below the maximum limit (Rosa et al., 2017). CO_2 and ethanol levels range from 0.08% to 0.20% (v/v) and 0.01% to 2.00% (v/v), respectively (Koroleva, 1988b; Farnworth, 2005; Gao and Li, 2016; Rosa et al., 2017).

The proximal composition of kefir is typically 0.7%–1.1% ash, 0.2%–3.5% fat, 3.0%–3.3% protein, 4.0%–6.0% sugar (lactose), and 80%–90% moisture (Otles and Cagindi, 2003; Arslan, 2015). The considerable variation in fat content is attributable to the fact that kefir is produced from whole milk, low-fat (partially skimmed) or fat-free (skimmed) milk (Sarkar, 2007; Arslan, 2015). However, whole milk is considered the ideal substrate (Gao and Li, 2016), since the higher fat content contributes to a thicker consistency and a creamier taste. Albeit that the composition of the kefir lipid content also varies with variations in the type of milk used, as well as with variations in composition of the grains (e.g., from different origins), it encompasses nonesterified fatty acids (NEFA), monoacylglycerols (MAG), diacylglycerols (DAG), triacylglycerols (TAG), and sterols. The presence of NEFA in kefir is said to contribute to enhanced digestibility (Rosa et al., 2017). Increases were observed for palmitic acid (a fatty acid with proven antimutagenic properties), as well as for monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), with a concomitant decrease in saturated fats during fermentation and storage (Vieira et al., 2015). *L. lactis* subsp. *cremoris* (isolated from Brazilian kefir), synthesized appreciable levels of conjugated linoleic acid (CLA) and PUFA, while at the same time reducing saturated fats, as well as the thrombogenic index in whole cow's milk fermented by this LAB (Vieira et al., 2017). CLA has desirable biofunctionalities, for example, antimutagenic and anticarcinogenic effects (Dos Reis et al., 2017; Rosa et al., 2017).

The fermentation also renders proteins more easily digestible, with elevated amino acid levels compared to milk. Increases occur during fermentation, and possibly during storage, in leucine, serine, tyrosine, L- and D-alanine, aspartic acid, L-cysteine, as well as the essential amino acids (in mg/100 g) tryptophan (50–80) (a precursor of serotonin in the body), kynurenic acid (a tryptophan derivative and a neuroprotective compound) (11–24), phenylalanine (231), isoleucine (210–262), L-threonine (170–183), methionine (137), lysine (270–376), and L- and D-valine (220) (Otles and Cagindi, 2003; Arslan, 2015; Bertazzo et al., 2016; Dallas et al., 2016; Menestrina et al., 2016; Rosa et al., 2017; Yılmaz and Gökmen, 2017). Although *de novo* amino acid synthesis is reported (Farnworth, 2005), most lactic acid bacteria (LAB) have limited capacity in this regard (Hugenholtz, 2008), hence the elevated amino acid levels in kefir are very likely due to the proteolytic activity of the LAB (Sarkar, 2007; Rattray and O'Connell, 2011; Ebner et al., 2015). Further testimony to the enhanced digestibility of kefir is the release of 609 protein fragments and alteration of the >1500 peptides derived from 27 milk proteins (Dallas et al., 2016), while 236 peptides were unique to kefir compared to 21 in milk. In addition, the peptides identified in kefir have molecular masses almost half that of milk, namely ≤2700 and ≤4400 Da, respectively (Ebner et al., 2015). Moreover, the enhanced protein digestibility combat against a potential inflammatory response evoked by putrefactive products produced in the GI in individuals with a compromised digestive system, particularly as concerns protein digestion (Dallas et al., 2016). Bioactive peptides (16–25) with putative antimicrobial, antihypertensive (angiotensin-converting enzyme [ACE]-inhibitory), opioid, antioxidative, antithrombotic, and immunomodulatory biofunctionalities were identified in kefir (Ebner et al., 2015; Dallas et al., 2016). The opioid functionality is of particular interest since it involves activation of intestinal opioid receptors which are capable of ameliorating visceral pain, one of the debilitating effects of inflammatory bowel disorders (IBDs) (Mizock, 2015). Ebner et al. (2015) made the interesting observation that the bioactive peptides in kefir made from commercial cultures (230) were 1.4-fold higher than in kefir made from grains (127), with 97 common to both. This opens the door to the possibility of using bioactive peptides as markers, common biopeptides to authenticate kefir products in general and variable biopeptides to differentiate between kefir varieties. The antimicrobial functionality of biopeptides is also of great interest. Recently, a novel antimicrobial peptide (F1) was elaborated from Tibetan kefir. It showed strong activity against *E. coli*, with the ability to disrupt the cell membrane, suggesting that it could have functionality against other pathogens (Miao et al., 2016).

While the vitamin composition is affected by factors like the quality of the milk, the quality and the composition of the grains, as well as the process, the kefir beverage is widely reported to contain several vitamins, including vitamins A, B_1, B_2, B_5, B_9 (folic acid), B_{12}, C, K, as well as carotene (Kneifel and Mayer, 1991; Otles and Cagindi, 2003; Sarkar, 2007; Van Wyk et al,. 2011; Arslan, 2015; Kesenkas et al., 2017; Rosa et al., 2017). Increases in vitamins B_6 (Kesenkas et al., 2017) and B_7 were also reported (Rosa et al., 2017). Kefir is a good source of the minerals calcium, magnesium, and phosphorus, while potassium is a further macroelement present in kefir. The microelements include zinc, copper, manganese, iron, molybdenum, and cobalt (Sarkar, 2007; Rosa et al., 2017).

The microbiota of the beverage comprises live organisms, albeit in lower numbers than in the grains, with estimations of lactobacilli at 10^7–10^8 CFU/g, lactococci, and *Leuconostoc* spp., each at 10^8–10^9 CFU/g and 10^5–10^7 CFU/g yeasts (Kroger, 1993; Rattray and O'Connell, 2011; Kalamaki and Angelidis, 2017) and 10^6 CFU/g acetic acid bacteria (Irigoyen et al., 2005), hence the lactococci and leuconostocs predominate in the beverage (Dobson et al., 2011; Marsh et al., 2013; Bourrie et al., 2016). The number of different organisms in the beverage is reported to exceed 50 (Kim et al., 2017), but, as is the case with the grains, various organisms are reported to predominate in the beverage. In the roughly 3000 year-old kefir sample analyzed by Yang et al. (2014), *Lb. kefiranofaciens* was identified as the dominant organism, while *L. lactis* (Dobson et al., 2011; Kesmen and Kacmaz, 2011; Porcellato et al., 2015), *L. mesenteroides* and *Leuconostoc raffinolactis* predominated in some batches (from the same supplier), indicating that considerable variation at species level may occur between different kefir beverages (Porcellato et al., 2015). Kim et al. (2016b) reported 10^4–10^6 CFU/mL *Lb. kefiri* and 10^5–10^7 CFU/mL *K. marxianus* (Kim et al., 2018) in the kefir beverages analyzed by them.

12.4.2 Factors That Influence the Quality of the Beverage

As described previously, using kefir grains rather than a grain-free starter, ensures a better quality beverage. The size of the inoculum was confirmed as another important factor that influences the quality of the final product (Garrote et al., 1998; Koroleva, 1988a; Walsh et al., 2016). Inoculum concentration spans a wide range, namely 0.1% (or 1:1000) (w/v) (Rea et al., 1996), 5% (or 1:20) (w/v) (Kroger, 1993), 10% (or 1:10) (w/v) (Van Wyk, 2002; Van Wyk et al., 2011), and even 20% (or 1:5) (w/v) (Marshall, 1984a), with a pragmatic approach most often governing the selection of inoculum concentration (Rosa et al., 2017). However, the preferred inoculum concentration of kefir grains, especially for industrial scale processes, is 2%–3% (or 1:50–1:30), since this results in the ideal pH as well as the ideal balance between the constituent microbiota of the beverage, that is, the lactobacilli, lactococci, and yeasts, thus producing the most palatable beverage in terms of flavor and consistency (Libudzisz and Piatkiewicz, 1990;

Rea et al., 1996; Garrote et al., 1998; Koroleva, 1988a,b; Rattray and O'Connell, 2011; Rosa et al., 2017). Agitation during fermentation favors the development of homofermentative lactococci and yeasts, thus also affecting the quality of the beverage (Rattray and O'Connell, 2011).

Most authors report that the optimum fermentation temperature is between 22°C and 25°C for optimum flavor and consistency of the finished product (Liu and Moon, 1983; Rosa et al., 2017), but 8°C (Rosa et al., 2017), 18°C (Kroger, 1993), 19°C (Libudzisz and Piatkiewicz, 1990), 20°C (Garrote et al., 1998), 21°C (Rea et al., 1996), 23°C, 28°C, and up to 30°C (Beshkova et al., 2002; Rattray and O'Connell, 2011) were also used. The fermentation times also vary from 10 to 40 h, but 24 h is the most common (Rosa et al., 2017).

Regularly rinsing the grains with potable or tap water results in an altered microbial population on the grain surface, negatively affecting the quality of the beverage (Koroleva, 1988a; Güzel-Seydim et al., 2005). However, weekly rinsing with sterile water or pasteurized skim milk is accepted practice during the commercial production process (Rattray and O'Connell, 2011).

Interaction of β-lactoglobulin with κ-casein may occur in fermented milk products, resulting in syneresis (Roginski, 1988). However, the heat treatment typically used for the pasteurization step affects denaturation of whey proteins, enhancing the consistency of and preventing syneresis in the beverage (Kroger, 1993; Kesenkas et al., 2017). Moreover, kefiran functions as a natural stabilizer in the beverage (Bensmira and Jiang, 2012; Bourrie et al., 2016; Rosa et al., 2017).

The CO_2 formed during the second fermentation may cause swelling or even blowing of containers at the distribution stage (Kwak et al., 1996). The storage at temperatures of 2–4°C and the use of suitable containers and closures, such as glass bottles sealed with crown caps, control the levels of CO_2 that are produced and also prevent distortion or blowing of containers (Duitschaever, 1989).

12.5 Kefir as a Functional or Probiotic Food

The health-promoting properties of kefir are not only well-documented, but it has been used as a traditional remedy for so long that it could be reasonably considered one of the oldest (Koroleva, 1988a; Vinderola et al., 2006b; Shavit, 2008; Rattray and O'Connell, 2011), if not the most ideal, probiotic dairy product (Heller, 2001). At present, there is immense interest in the potential of kefir and/or its constituent organisms as biofunctional foods, particularly its probiotic functionalities (Carasi et al., 2015). A wide array of health benefits, including prophylactic, therapeutic, and physiological benefits, is attributed to kefir consumption. The longevity of Caucasian people had been ascribed to kefir consumption (Garrote et al., 2000), while in Tibet and environs it is not only a popular health-promoting beverage, but is considered a natural folk remedy (Diniz et al., 2003). Moreover, in Russia and other former Soviet countries, as well as in countries inhabited by immigrants from Eastern Europe (e.g., Argentina) kefir is also a popular staple and its health-promoting and prophylactic properties are widely accepted (Koroleva, 1988a; Garrote et al., 2001; Vinderola et al., 2005, 2006b). In these countries, the historical practice of kefir being recommended by physicians continues until the present day. In this context, kefir is known to reduce the risk of chronic disease in healthy individuals, while it is a prescribed therapy for patients suffering allergies, hypertension, ischemic heart disease, metabolic and gastrointestinal (GI) complaints, and being overweight (Koroleva, 1988a; Garrote et al., 2001; Zubillaga et al., 2001; Shavit, 2008; Rattray and O'Connell, 2011; Atanassova et al., 2014; Kim et al., 2016a; Rosa et al., 2017; Santanna et al., 2017). Further beneficial effects of kefir consumption include antimicrobial, immunomodulation and attendant antiinflammatory effects, antitumoral as well as hypocholesterolemic effects (Liu et al., 2002b; Carasi et al., 2015; Rosa et al., 2017).

The range of health-promoting effects attributed to kefir is closely associated with its probiotic effects (Vinderola et al., 2006b; Hong et al., 2009; Cousin et al., 2011; Karimi et al., 2011). The probiotic effects are firstly ascribed to the direct effects of the live constituent microorganisms with recognized probiotic potential, that is, with confirmed resistance to bile salts and the low pH of the GI tract, as well as proven ability to adhere to the gastric mucosa (Vinderola et al., 2006b; Carasi et al., 2014b; Bengoa et al., 2017; Rosa et al., 2017). The second probiotic mechanism involves the immunomodulatory effects, including the indirect beneficial effects of metabolites of the microorganisms present in the consortium, notably peptides (biogenics) (Vinderola et al., 2006b; Dallas et al., 2016). However, a third aspect of the probiotic effect is the fact that the kefir microbiota produce antimicrobial metabolites (including organic acids, hydrogen peroxide, and bacteriocins) which inhibit pathogens, particularly in the intestinal mucosa (Rattray and O'Connell, 2011; Rosa et al., 2017). In addition, kefiran displays antimicrobial activity, and being resistant to hydrolysis by bowel enzymes, is therefore a good candidate for contributing to the probiotic properties of kefir (Rosa et al., 2017).

The probiotic effect of live microorganisms firstly concerns their role in maintaining intestinal homeostasis by preventing or correcting dysbiosis, that is, by modulating the balance between beneficial commensal bacteria and harmful enteric pathogens and, by extension, the interactions between the human microbiota, intestinal epithelial cells, and immune cells (Carasi et al., 2015; Mizock, 2015; Vandenplas et al., 2015; Toscano et al., 2017; Yazdi et al., 2017). After colonization and interaction with the mucous microbiota and acting primarily in the gut-associated lymphoid tissue (GALT), the probiotic microorganisms evoke the expression and release of immune mediators such as chemokines and anti- or pro-inflammatory cytokines which play a role in regulating both the innate and adaptive immune response. Hence, by modulating the intestinal microbiota, probiotics have an immunomodulating role, both at mucosal and systemic level. This opens possibilities for their use as alternative treatments to prevent and/or combat different immunopathologies (such as IBD and allergies), chronic diseases (such as obesity, cancer, and diabetes), as well as intestinal infections caused by pathogens (such as *Salmonella enterica*) (Liu et al., 2006b; Carasi et al., 2015; De Araujo et al., 2017; Toscano et al., 2017).

The results of the administration of kefir-based probiotic preparations to human subjects reinforced the tenet that it shows great promise as a probiotic. *Lb. kefiri* LKF01 (DSM32079), isolated from water kefir grains, was recently administered as an oral probiotic to 20 Italian volunteers (Toscano et al., 2017). Strains of this organism are safe for human consumption (Carasi et al., 2014a, 2015), demonstrated acid and bile tolerance at concentrations prevalent in the small intestine (Yazdi et al., 2017) and retained viability, as well as the ability to adhere to mucosal cells, after freeze drying or spray drying, confirming its suitability as a probiotic (Carasi et al., 2014a, 2015; Huang et al., 2017; Yazdi et al., 2017). Toscano et al. (2017) demonstrated that it was present in the feces of the subjects 1 month after cessation of administration, confirming its ability to adhere to and colonize the GI (Carasi et al., 2015; Toscano et al., 2017). The composition of the intestinal microbiota was changed by the probiotic organism, but the microbial diversity retained the desired complexity in order to continue to play a role in combatting the onset or progression of chronic disorders, like obesity, and of immunopathologies, like irritable bowel syndrome (IBS). Some of the most noteworthy beneficial changes to the gut microbiota included a significant reduction of the phylum *Proteobacteria* and species of the genera *Clostridium*, *Staphylococcus*, *Veillonella*, and *Salmonella*. The reduction in *Proteobacteria* is of special significance since its prevalence leads to dysbiosis which was shown to make the individual more prone to disorders like eczema, asthma, and rhinoconjunctivitis (atopic disorders), obesity, diabetes, and IBS. *Clostridium*, *Streptococcus*, and *Salmonella* are among those organisms which are capable of evoking a negative immunomodulatory effect, leading to a severe inflammatory response. Hence, the reduction in these undesirable organisms pointed to strong antiinflammatory activity of *L. kefiri* LKF01. *Streptococcus* and *Veillonella*, in particular, show strong metabolic interaction in the intestinal microbiota, leading to a more pronounced proinflammatory response, thus precipitating the onset of

IBD and other enteric disorders. Furthermore, *L. kefiri* LKF01 did not adversely affect the beneficial commensal bacterium *Faecalibacterium prausnitzii*, which synthesizes butyrate and other short-chain fatty acids (SCFAs), thus promoting an antiinflammatory response, protecting the host against enteric disorders like IBD (Mizock, 2015; Toscano et al., 2017), which manifests either as Crohn's disease or as ulcerative colitis. Thus far, probiotics proved effective against ulcerative colitis, but not against Crohn's disease (Carasi et al., 2015; Mizock, 2015). In addition to that of Toscano et al. (2017), other studies that demonstrated altered gut microbiota in response to kefir administration are reported. Oral administration of kefir to mice resulted in a reduction of *Clostridium* and *Enterobacteriaceae* in the GI, while a significant increase in *Lactobacillus* and *Bifidobacterium*, concomitant with reduced *Clostridium perfringens* numbers in the feces was also observed (Liu et al., 2006b). A *Lb. plantarum* strain isolated from kefir protected mice against intestinal infection by *Yersinia enterocolitica*, while exerting an immunomodulatory effect (Montijo-Prieto et al., 2015). Increased fecal numbers of *Lactobacillus* were also observed in murine models in response to administration of a *Lb. kefiri* strain (Carasi et al., 2015). Kefiran stimulated the growth of *Bifidobacterium* in vitro (Serafini et al., 2014; Piermaria et al., 2015), indicating its potential to do likewise in the GI, considering that it is resistant to enzymes in the intestine (Rosa et al., 2017). Moreover, kefir contains organisms with proven probiotic status; a number of *Bifidobacterium* spp., including *B. longum* was found in kefir samples (grains and beverage) (Dobson et al., 2011; Taş et al., 2012), while the presence of *Lactobacillus acidophilus* (Vasiljevic and Shah, 2008; Dobson et al., 2011) and that of *Lb. kefiri* is well-established (Anon., 2003; Carasi et al., 2015; Demetri et al., 2017; Toscano et al., 2017). In addition to these two LAB, the kefir consortium potentially contains a further 16 strains with widely accepted probiotic status (Table 12.1) (Illanes and Guerrero, 2016), with genes encoding for probiotic functionalities detected in the kefir microbiota (Walsh et al., 2016), further illustrating the probiotic potential of kefir. Derived from a kefir probiotic drink, *Lb. kefiri* was recently successfully encapsulated in alginate microbeads, thus protecting the probiotic organism against environmental adversity, including the gastric environment, ensuring maximum viability when it reaches the colon, able to exert its probiotic functionality in the colon (Demetri et al., 2017).

The vital role of immunomodulation in health pivots around the fact that the immune response is the first line of defense in protecting the body against pathogens in the tissues and in circulation (Vinderola et al., 2006b). The consequence of dysbiosis in terms of negative immunomodulation, followed by inflammatory responses, was highlighted in the preceding sections. The obverse is that probiotics restore homeostasis of the intestinal microbiota and thus orchestrate positive immunomodulation as well as antiinflammatory responses (Cousin et al., 2011; Karimi et al., 2011; Carasi et al., 2015). In this context, the essence of the probiotic effect could be considered to be the ability of the beneficial organism(s) to interact with the immune system to effect the establishment and maintenance of a balance in pro- and antiinflammatory cytokines (Carasi et al., 2015). Focusing on this aspect, many studies report the ability of kefir and kefir-derived preparations to attenuate negative immunomodulation, the consequent inflammatory responses, as well as the disorders precipitated by that (Carasi et al., 2015; Rosa et al., 2017). In this context, in response to orally administered kefiran (Vinderola et al., 2006a), as well as fresh, pasteurized and reconstituted freeze-dried kefir fractions, in vivo mucosal immunomodulation had been demonstrated in mice, confirming this aspect of the probiotic effect of kefir (Vinderola et al., 2005, 2006b). By virtue of its antimicrobial activity (Rosa et al., 2017), kefiran also demonstrated a probiotic effect by protecting Caco-2 cells (cultured human enterocytes) against extracellular toxin-induced damage by *Bacillus cereus* B10502 (Medrano et al., 2008; John and Deeseenthum, 2015). This observation suggested a novel mechanism for the protective effect of kefir as a probiotic in the GI, namely that its component kefiran demonstrated the ability to disrupt key events in the virulence of *B. cereus*, a spore-forming intestinal pathogen that produces cytopathic effects (Rimada and Abraham, 2006). Moreover, kefiran also demonstrated immunomodulatory activity in vitro and in vivo (Piermaria et al., 2015). One of the most important strains in kefir, *Lb. kefiri*, has the ability to antagonize the adhesion to and invasion of epithelial cells (Caco-2 cells) by *Salmonella enterica* serovar Enteritidis (*S. enteritidis*), the pathogen responsible for the majority of human intestinal infections (Golowczyc et al., 2007; Londero et al., 2015). *Salmonella typhimurium* and *Escherichia coli* are also major enteropathogens responsible for typhoid fever and gastroenteritis, respectively. These pathogens also invade epithelial cells, causing inflammation which culminates in epithelial lesions. *Lactobacillus acidophilus*, another member of the kefir consortium, demonstrated antagonization of these two pathogens (Meng et al., 2017). The protection mechanism was shown to be the antagonization of pathogen invasion of the enterocytes (which occurs via interaction with and subsequent disorganization of the microvilli of intestinal brush border cells) by SLP secreted by *Lb. kefiri* (Golowczyc et al., 2007; Cavallero et al., 2017) and *Lb. acidophilus* (Meng et al., 2017). The SLP from *Lb. kefiri* contain *O*-glycosylated peptides as well as peptides with short *N*-glycan chains, which may pave the way toward greater understanding of the role of SLPs in antagonizing the adverse effects of different enteric pathogens (Cavallero et al., 2017). Furthermore, in an in vivo study in mice, Carasi et al. (2015) demonstrated that *Lb. kefiri* strain CIDCA 8348 evoked downregulation of proinflammatory mediators (IFN-γ, GM-CSF, and IL-1β genes), while attenuating the production of proinflammatory cytokines (such as IL-6 and GM-CSF) and increasing antiinflammatory cytokines (IL-10) in the intestinal immune system, as well as in the ileum and colon. Ileum and colon explants further demonstrated the antiinflammatory effect by showing reduced levels of IL-6 and GM-CSF in mice treated with *Lb. kefiri*, compared to control mice. Kefir yeasts *K. marxianus* and *S. cerevisiae* also demonstrated the downregulation of the intestinal innate immune response (Carasi et al., 2015).

Kefir was shown to alleviate various manifestations of allergy of which the clinical classification includes entities like food allergies, allergic asthma, atopic dermatitis/eczema, and allergic rhinitis (Mizock, 2015). The administration of milk-based, soymilk-based (Liu et al., 2006b), and freeze-dried kefir (Umeda et al., 2005) to mice where the allergen-specific IgE response was induced by ovalbumin (OVA), significantly reduced the levels of IgE and IgG1 in the serum and suppressed the responses typical of these two immunoglobulins, that is, typical of an allergic reaction (Liu et al., 2006b). The observed immunomodulatory effects were attributed to the ability of the probiotic to strengthen the GI barrier, thus reducing intestinal permeation of, and the associated suppression of oral sensitization to, food-borne antigens or allergens. These observations highlighted the promise that kefir holds toward preventing food allergies (Umeda et al., 2005; Liu et al., 2006b). Furthermore, in an in vitro study, both *Lb. kefiranofaciens* extracted from kefir and its supernatant attenuated production of proinflammatory cytokines (Hong et al., 2009). Consequently, when kefir administration resulted in antiasthmatic and antiinflammatory effects in mice sensitized and hence allergic to ovalbumin (Lee et al., 2007), as well as in the amelioration of colitis in dextran sulfate sodium (DSS)-induced mice, the therapeutic effects were attributed to the presence of *Lb. kefiranofaciens* in the kefir (Carasi et al., 2015). In human subjects (infants), kefir administration could not be linked conclusively to amelioration of a respiratory condition called infant wheezing, but the results showed positive immunomodulation, indicating that more research in this direction is warranted (De Araujo et al., 2017).

In addition to the immunomodulatory effects of the microbiota and kefiran, 25 bioactive peptides formed in kefir during the fermentation have also been shown to promote the cell-mediated immune response against intracellular pathogens and infections (Carasi et al., 2015; Dallas et al., 2016; Rosa et al., 2017). Hence, the aforementioned studies indicate the functionality of kefir and its fractions as biotherapeutic agents capable of positive immunomodulation of the GI immune system, but also the immune system of the host, including cell-mediated immune responses against allergens, antigens, tumors, as well as against intercellular pathogenic infections (Vinderola et al., 2006b, Hong et al., 2009; Carasi et al., 2015; Rosa et al., 2017; Toscano et al., 2017).

Compelling evidence exists for the association between colorectal cancer and intestinal dysbiosis (Mizock, 2015). This resonates with the fact that the positive modulation of the host's immune system is also considered to be a key factor in the prevention and attenuation of carcinogenesis by kefir (De Araujo et al., 2017; Dos Reis et al., 2017; Kesenkas et al., 2017; Rosa et al., 2017). Moreover, kefir also acted in other anticarcinogenic roles, it namely inhibited proliferation of subcutaneous carcinoma in mice (Sarkar, 2007) and caused apoptosis of colorectal cancer cells (Mizock, 2015; Dos Reis et al., 2017; Rosa et al., 2017), while milk-kefir and soymilk-kefir demonstrated significant antioxidant and antimutagenic properties (Liu et al., 2005) and, lastly, kefir demonstrated inhibition of enzymes that convert procarcinogenic compounds to carcinogens (Kesenkas et al., 2017). In terms of antimutagenic activity, *Lb. kefiri* and *Kazachstania servazzii*, isolated from kefir were able to bind 98%–100% mutagenic substances, notably aflatoxin B1, zearalenone, and ochratoxin (Rosa et al., 2017; Taheur et al., 2017). Since the complexed mutagens are then excreted the kefir organisms effectively protect colon epithelial cells from damage. The antimutagenic effects against mutagens like sodium azide, aflatoxin B1, and methyl methane-sulfonate were also linked to relatively high levels of CLA isomers, butyric, oleic, and palmitoleic acids in kefir (Rosa et al., 2017), while CLA and butyrate are also proven anticarcinogens (Dos Reis et al., 2017). Kefir supernatants also demonstrated antigenotoxic effects by decreasing fecal water-induced DNA damage to colon cells in vitro (Grishina et al., 2011). Hence, regular kefir consumption may mitigate against the risk for colorectal cancer (Rosa et al., 2017). Anticarcinogenic, including antitumor, effects of kefir and its constituents had also been demonstrated for other cancer types, namely breast cancer (in a murine model), leukemia and lymphoma, as well as sarcoma (connective tissue tumor) (Kesenkas et al., 2017; Rosa et al., 2017). Bioactive compounds of kefir also showed protective effects against pulmonary metastasis and inhibited melanoma metastasis. As stated, kefir also demonstrates significant antioxidant activity (Liu et al., 2005; Leite et al., 2015), with specific antioxidant functionality ascribed to constituents such as biopeptides (Ebner et al., 2015) and kefiran (Prado et al., 2015; Nampoothiri et al., 2017). The significance of the antioxidant effect is evident when considering that accumulation of reactive oxygen species (ROS) that are continuously produced during normal metabolism, cause damage to DNA and other macromolecules, potentially culminating in diseases such as cancer, arthritis, and atherosclerosis. Hence, due to its proven antioxidant capacity, kefir is therapeutic by virtue of its ability to inactivate ROS (Nampoothiri et al., 2017), as demonstrated when orally administered kefir prevented ethanol-induced gastric ulcers in γ-irradiated rats (Fahmy and Ismail, 2015).

The potential probiotic effect in terms of inhibition of growth and virulence of pathogens in the GI, highlighted in a previous section, is attributable to the considerable antibacterial and antifungal activity of kefir. This aspect of kefir has fuelled extensive research interest of late (Yüksedağ et al., 2004; Carasi et al., 2015; Miao et al., 2015a,b; Kim et al., 2016a; Kesenkas et al., 2017; Rosa et al., 2017), while it also evokes interest from a food safety point of view (Brialy et al., 1995; Yüksedağ et al., 2004; Miao et al., 2015a, 2016). The kefir culture, the kefir beverage, and constituent organisms display widely acknowledged antibacterial activity against many pathogens and coliforms (John and Deeseenthum, 2015; Rosa et al., 2017). Bacteriostatic and bacteriocidal effects are exerted against both Gram-negative and Gram-positive organisms, but greater bacteriocidal activity is prevalent against Gram-positive bacteria (Sarkar, 2007; Rattray and O'Connell, 2011; John and Deeseenthum, 2015; Rosa et al., 2017). The antibacterial activity is attributed to a number of factors, ranging from competition for available nutrients to the inhibitory action of metabolites of the kefir consortium, namely organic acids, hydrogen peroxide, acetaldehyde, carbon dioxide, bacteriocins (John and Deeseenthum, 2015; Mizock, 2015; Rosa et al., 2017), diacetyl, acetaldehyde (Powell et al., 2007; Kesenkas et al., 2017), and bioactive peptides (Mizock, 2015; Dallas et al., 2016). Hippuric acid, one of the organic acids, offers the tantalizing possibility that it may be converted to benzoic acid by LAB, thus contributing to the preservative effect (Heller, 2001). Benzoic acid with levels of 8–23 mg/kg has been reported in kefir (Sieber et al., 1995). Hydrogen peroxide (H_2O_2) is a powerful antimicrobial agent, but the inhibition of *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* by kefir *Lactococcus* strains could not be correlated with the H_2O_2 (0.00–0.17 µg/mL) produced by these strains (Yüksedağ et al., 2004). Instead, it is more than likely that the inhibition was due to the synergism between the well-known bacteriostatic effect of the undissociated lactic (4.9–9.2 mg/mL) and acetic acids, combined with the effects of the H_2O_2 and acetaldehyde (1.14–3.96 µg/mL), as well the proteolytic activity (0.00–0.05 mg/mL), in other words that antagonism and/or preservation is achieved via a hurdle effect (Garrote et al., 2000; Yüksedağ et al., 2004; Rattray and O'Connell, 2011; Kim et al., 2016a). Administration of Lifeway kefir to patients with recurrent CDI, along with staggered and tapered antibiotic withdrawal (STAW), resulted in complete resolution of symptoms in 84% of the subjects, highlighting the potential of kefir as an effective natural alternative antimicrobial to synthetic antibiotics, offering a possible solution to the escalating antimicrobial resistance crisis (Spinler et al., 2016b). Bacteriocins are also credited with contributing toward the observed antimicrobial activity. Lacticin 3147, a bacteriocin produced by *L. lactis* strain DPC3147, isolated from kefir grains, demonstrated antimicrobial activity against *Y. enterocolitica*, *Listeria monocytogenes*, *S. typhimurium*, *S. enteritidis*, and *Shigella flexneri* (Zubillaga et al., 2001; Dobson et al., 2011). *Lb. plantarum* ST8KF, another organism found in the kefir consortium, produces a 3.5 kDa bacteriocin with bacteriostatic activity against several LAB, as well as against *Listeria innocua* (Powell et al., 2007). The bacteriocin with the widest antagonistic spectrum thus far was elaborated by *Lb. paracasei* subsp. *tolerans* FX-6, a probiotic strain isolated from Tibetan kefir. Not only Gram-negative bacteria (*E. coli*, *Salmonella enterica* and *Shigella dysenteriae*) and Gram-positive bacteria (*S. aureus* and *Bacillus thuringiensis*), but also fungi (*Aspergillus flavus*, *A. niger*, *Rhizopus nigricans* and *Penicillium glaucum*) were inhibited (Miao et al., 2015a). In addition to the aforementioned organisms, inhibition of a wide spectrum of other bacteria by kefir and kefir components is reported, namely *Klebsiella pneumoniae*, (Brialy et al., 1995; Garrote et al., 2000; Yüksedağ et al., 2004), *Bacillus cereus* (Medrano et al., 2008), *Bacillus subtilis*, *Salmonella typhi*, *Helicobacter pylori*, *Shigella sonnei*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Streptococcus pyogenes*, *Micrococcus luteus*, *Micrococcus flavans* (Rattray and O'Connell, 2011; Rosa et al., 2017), *Enterococcus faecalis* (Chifiriuc et al., 2011; Carasi et al., 2014b) and *Cronobacter sakazakii* (Kim et al., 2016a). In some cases, the antibacterial effect of kefir exceeded that of antibiotics like ampicillin and neomycin (Chifiriuc et al., 2011). Antimycotic activity of kefir against yeast and filamentous fungi was also observed. Inhibition of *Candida albicans*, a well-known GI pathogen (Vasiljevic and Shah, 2008; John and Deeseenthum, 2015), *Torulopsis glabrata*, *Microsporum nanum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Aspergillus ochraceus*, as well as inhibition of *Fusarium graminearum* and the complete inhibition of sporulation of *A. flavus* (both filamentous fungi) was observed. The significance of the latter is that production of aflatoxin B1 is thus prevented (Kesenkas et al., 2017; Rosa et al., 2017).

It needs to be noted that the antimicrobial activity is differentiated for different formats of kefir, in other words, a different spectrum of activity is observed between the fresh and reconstituted kefir beverage, extracted kefiran and kefir grains, offering the possibility of tailor-made antimicrobial kefir-based preparations (Vasiljevic and Shah, 2008; John and Deeseenthum, 2015; Rosa et al., 2017).

The direct association with excessive cholesterol consumption and cardiovascular disease exists, establishing it as a significant public health concern (Sarkar, 2007) and consequently stimulating interest in kefir which has the potential to be manufactured as a low-cholesterol product, ascribed to the activity of a cholesterol degrading enzyme (Vujicic et al., 1992; Bonczar et al., 2016). However, the possibility that kefir has therapeutic properties in terms of reduction of serum cholesterol levels is an even more tantalizing prospect (Farnworth, 2005; Kesenkas et al., 2017), fuelling research interest in this area (Rosa et al., 2017). The fact that the results of most of the in vitro and in vivo studies indicated that consumption of fermented dairy products has a hypocholesterolemic effect (Kesenkas et al., 2017) and that probiotic dairy products were shown to lower serum cholesterol, LDL cholesterol and TAG concentrations (Rosa et al., 2017) stimulate further interest. The hypocholesterolemic and/or antiatherogenic effects of kefir and kefiran were demonstrated when administered to hamsters (Liu et al., 2006a), mice, or rabbits fed on hypercholesterolemic or hyperlipidemic diets (Rosa et al., 2017), while vascular lipid deposition was reduced in low-density lipoprotein receptor-deficient mice, reinforcing the protective role of kefir and its potential as a prophylactic treatment against atherosclerosis (Santanna et al., 2017). *K. marxianus* (a yeast present in the kefir microbiota) (Yoshida et al., 2004) and kefiran from *Lb. kefiranofaciens* (Kesenkas et al., 2017) demonstrated significant hypocholesterolemic effects in rats on a high cholesterol diet, while other LAB that demonstrated cholesterol-reducing effects include *Lb. kefiri*, *Lb. acidophilus*, and *Lb. plantarum*. The bile-salt hydrolase activity observed in *K. marxianus* is the most likely mechanism responsible for their cholesterol attenuation activity (Kesenkas et al., 2017). However, these effects were not conclusively demonstrated in human subjects: while SCFA concentrations in the feces of hypercholesterolemic adult men were increased, kefir consumption by the test subjects did not have a therapeutic effect on cholesterol or plasma lipid concentrations (Rosa et al., 2017). Although kefir did not have a significant effect compared to low-fat milk, promising results were obtained when significant reduction in serum levels and ratios of lipoproteins were observed after kefir consumption by overweight or obese women, compared to the control cohort. Results included significantly lower TAG, total cholesterol, and low- and high-density lipoproteins, indicating that kefir consumption indeed has potential as therapy against hypercholesterolemia (Fathi et al., 2017). Kefir administration also significantly alleviated obesity and nonalcoholic fatty liver disease lesions in mice, accompanied by a reduction in IL-6 plasma levels, suggesting attenuation of inflammation via modulation of the gut microbiota by kefir (Kim et al., 2017).

The regular consumption of probiotics, and kefir in particular, was shown to control glycemia in rats (induced to diabetes mellitus), as well as in diabetic human subjects (Samah et al., 2016; Rosa et al., 2017).

Administration of *Lb. mali* isolated from kefir resulted in glucose homeostasis in diet-induced obese mice (Lin et al., 2016), while kefir consumption increased glucose uptake in skeletal muscle cells. The latter observation shows that kefir has significant potential toward decreasing insulin resistance in type-II diabetics (Kesenkas et al., 2017). Hence, kefir may be therapeutic toward controlling diabetes, but more work is required toward producing conclusive evidence (Kesenkas et al., 2017; Rosa et al., 2017). There is also merit in pursuing further studies to probe the purported antihypertensive capacity of kefir, since kefir contains at least 25 bioactive peptides, the majority of which are ACE inhibitors (Ebner et al., 2015; Dallas et al., 2016; Rosa et al., 2017), while antihypertensive activity was also attributed to the ability of kefiran to inhibit ACE activity (Kesenkas et al., 2017; Rosa et al., 2017).

The kefir grains and beverage contains β-galactosidase, an enzyme that is also present in the human GI tract, where its role is hydrolysis of lactose to facilitate its normal digestion in the small intestine. However, 75% of the global population either lacks or experience diminished activity of this enzyme, resulting in the digestive disorder known as lactose intolerance which manifests upon consumption of unfermented dairy products. Consumption of kefir by these individuals was demonstrated to have a prophylactic effect in terms of this disorder (Koroleva, 1988b; Farnworth, 2005; Vasiljevic and Shah, 2008; Rattray and O'Connell, 2011; Rosa et al., 2017). The prophylactic effect is ascribed to the reduced lactose levels in kefir, but it may also relate to reduced gastric emptying (Hertzler and Clancy, 2003), as well as enzymes released from microorganisms lyzed in the GI and hence is considered another facet of the probiotic effect (Vasiljevic and Shah, 2008). The reduced lactose content also makes the kefir beverage more suitable for diabetics (Rosa et al., 2017).

Novel approaches toward exploiting the probiotic effect include topical applications (Rosa et al., 2017). In this regard, kefir demonstrated both antibiotic and cicatrizing (wound-healing) properties. When applying a 70% kefir gel prepared from ground grains, as well as a pure kefiran gel, to cutaneous wounds in rats, infected with *S. aureus*, inhibition of the preparations against various pathogens and their cicatrizing effect were recorded. Kefiran was the most effective against *S. pyogenes*, followed by *S. aureus* and *Streptococcus salivarius*, while the kefir suspension inhibited *P. aeruginosa*, *S. aureus*, *L. monocytogenes*, *E. coli*, *C. albicans* and *S. pyogenes* more effectively than the antibiotics used in the study. The kefir gel also resulted in a faster reduction of the wound diameter than a neomycin-clostebol preparation, thus confirming its potential as a cicatrizing agent (Rodrigues et al., 2005). Gels containing cell-free kefir supernatants also produced a cicatrizing effect when applied to burn wounds in rats infected with *P. aeruginosa*, with significantly higher epithelialization and lower inflammation than an antibiotic preparation after 2 weeks (Huseini et al., 2012). The curative effects of these kefir preparations (Rodrigues et al., 2005), as well as that of cell-free kefir supernatants (Huseini et al., 2012; Tsiouris et al., 2017) are clearly related to its antimicrobial capacity, but its superior activity toward wound diameter reduction, compared to antibiotics, indicate that it is able to exert an antiinflammatory effect in this milieu and not only in the GI tract.

12.6 Other Applications and Potential Applications of Kefir and Kefir-Based Components in the Food Industry

Kefir and kefir grains were also used as starters in several other food products. It was used to produce cheese (Katechaki et al., 2009; Gao and Li, 2016) and a good quality labneh (a thick yoghurt or soft cheese) (Rocha et al., 2014). Grain-free preparations (fresh, thermally, or freeze-dried kefir) are preferred to grains since the use of the latter is impractical due to handling problems during transportation, storage, and standardization of inoculum concentration. Not only did the kefir inoculum produce cheese with superior aroma, taste, and texture, but it also had a longer shelf life. This offers an added incentive for cheese producers to use kefir inocula in cheese, since its antimicrobial functionalities reduce or even obviate the need to use chemical preservatives (Katechaki et al., 2009). Kefir grains had also been used as a leavening agent to replace traditional baker’s yeast or sourdough starters. The bread had good loaf volume, was moister (Nielsen et al., 2014), had a longer shelf life, in terms of delayed staling, mold spoilage, and rope formation by *Bacillus subtilis* (Plessas et al., 2005; Mantzourani et al., 2014). The bread made with 20% w/w kefir (flour basis) showed a more complex profile of volatiles, with lower rates of volatile losses during storage. This likely contributed to its significant preference by a consumer panel to bread made with only 10% w/w kefir (Plessas et al., 2011) or with traditional baker's yeast (Plessas et al., 2005), indicating the suitability of kefir grains in this application. Kefir grains were also used to produce apple-based vinegar (Viana et al., 2017), malt-based beer (Rodrigues et al., 2016), while immobilized and free kefir cultures effected simultaneous alcoholic and malolactic fermentations, producing high-quality ciders (Nikolaou et al., 2017).

Kefiran was successfully employed to prepare various films. Incorporating glycerol as a plasticizer to prepare a kefiran-based biodegradable edible film increased the extensibility, but decreased tensile strength and increased water vapor permeability (Ghasemlou et al., 2011). Piermaria et al. (2015) developed a novel delivery system for probiotic organisms isolated from kefir and enveloped by edible kefiran films containing glycerol as plasticizer. Both strains, *Lb. plantarum* CIDCA 8327 and *K. marxianus* CIDCA 8154 retained their viability during storage, obviating the necessity for cold storage. Using ZnO nanoparticles (Shahabi-Ghahfarrokhi et al., 2015) or chitosan (Sabaghi et al., 2015) to replace carbohydrate-based plasticizers, films with superior tensile strength and moisture-barrier capacity were produced. Incorporation of ZnO nanoparticles also achieved UV protection, while chitosan imparted antioxidant activity, hence producing kefiran films with food packaging potential (Shahabi-Ghahfarrokhi et al., 2015; Sabaghi et al., 2015). Kefiran was also combined with alginate to produce microspheres for the oral delivery of the antibiotic ciprofloxacin, resulting in a synergistic antimicrobial effect against several pathogens (Blandón et al., 2016).

Kefiran acts as a stabilizer in kefir (Bensmira and Jiang, 2012), suggesting that pure kefiran has potential as a food additive in other food products, particularly dairy products (Hussain et al., 2017). Indeed, kefiran (99% purity) used in skim-milk gels demonstrated its potential as a natural viscosifying agent in dairy products, with both viscosity and viscoelasticity enhanced, in milk acidified with glucono-δ-lactone (Rimada and Abraham, 2006) or when the acidification occurred during fermentation of milk with LAB strains isolated from kefir (Hamet et al., 2015). However, although kefiran solutions demonstrated flow behavior similar to other neutral hydrocolloids (methylcellulose, guar, and locust bean gum), it yielded lower viscosities at similar concentrations, indicating the possible need to combine it with other viscosifying agents in food formulations (Piermaria et al., 2016). On the other hand, *Lb. paracasei* strains isolated from kefir produced a high M_w hydrocolloid, resulting in acid milk gels with higher viscosity (Hamet et al., 2015). Moreover, kefiran solutions displayed superior emulsifying capacity than xanthan, guar, and locust bean gum (Güzel-Seydim et al., 2010) and also form gels after freezing, adding to its attractiveness as an alternative texturizing agent in food applications (Piermaria et al., 2008).

Kefiran is, therefore, clearly a valuable commodity, meriting investigation into processes that will maximize its production yield. Dailin et al. (2016) described a cultivation medium as well as semi-industrial scale process conditions for *Lb. kefiranofaciens* that resulted in a yield of 1.91 g/L kefiran, translating into a 58% increase in the yield. Cheese/casein whey can be used as the carbon source (Rimada and Abraham, 2001), leading to valorization of this high concentration waste water pollutant (Jiang et al., 2015).

In response to the increasing consumer demand for natural or biopreservatives, antimicrobials produced by *Lb. paracasei* subsp. *tolerans* FX-6 (isolated from kefir) were investigated. A bacteriocin (F1) elaborated by this organism showed great potential as a natural preservative in pork, extending the shelf life by 12 d (Miao et al., 2015a). In addition to F1, these authors identified two further fractions with antimicrobial activity produced by *Lb. paracasei* subsp. *tolerans* FX-6, which may also be bacteriocins. They demonstrated that bacteriocin F1, as well as another fraction, displayed stronger antimicrobial activity than nisin, confirming the great promise that the antimicrobials produced by *Lb. paracasei* subsp. *tolerans* FX-6 holds toward producing preservatives with clean-label status (Miao et al., 2015b). The kefir beverage also inhibited fungal spoilage of corn arepas, thus indicating its suitability to replace potassium sorbate in this application (Gamba et al., 2016).

Kefir grains are also useful vehicles for including microorganisms that are good producers of specific vitamins. In this regard, elevated folate and vitamin B_{12} content of the kefir beverage resulting from kefir grains treated with a *Propionibacterium freudenreichii* strain (strain J19) (Van Wyk et al., 2011), as well as *S. thermophilus* (ATCC 19258) (unpublished results) had been achieved, attaining at least 100% of the Nutrient Reference Values (NRV) per 100 mL serving of the kefir.

12.7 Conclusion

The potential of kefir as a biofunctional food due to its rich and varied microbiota and their production of bioactive compounds is undoubted. An overwhelming volume of current research into various facets of kefir is being conducted. The employment of increasingly evolving culture-independent techniques, including various OMIC-approaches, makes complete elucidation of the kefir microbiota, its genetics, its metabolics, and its interactions (both symbiotic and with the environment, i.e., the fermentation conditions) a credible target. Another burgeoning field of investigation is the putative health effects of kefir and its components, with advances in new methods producing mounting evidence that supports these putative health benefits. However, considerable work is still required toward: (1) application of new and emerging culture-independent and culture-dependent methods to achieve a complete description the composition of the grains, as well as the driving forces behind set patterns of microbial succession and the associated metabolite production; (2) accurate definition of the effects of the production method on the composition and characteristics of both the grains and the beverage; and (3) extensive experimental and clinical studies to completely describe the physiological, prophylactic, and therapeutic properties of kefir grains and its constituents. This is feasible, considering that, using a systems biology approach, combining metabolomics and metagenomics, Walsh et al. (2016) demonstrated that not only could they identify the constituent microorganisms in the microbiota in the grains and the beverage, but also their relative predominance and the metabolites associated with them. In the process, greater insight was gained into the dynamics of microbial succession, opening the door toward future optimization of the (1) microbiota (perhaps in the format of multistrain starter cultures with the same quality as the kefir grains, possibly immobilized in a kefiran film; (2) the fermentation process; (3) the flavor, consistency, and other quality aspects of the beverage; as well as (4) the health-promoting functionalities. Ponomarova et al. (2017) exploited several omic techniques to gain insight into metabolic fluxes and regulatory processing. These results suggest a potential route toward formulating the ideal kefir culture and toward engineering predictable kefir fermentations in the future. Moreover, complete elucidation of the kefir microbiota and deeper understanding of its probiotic effects, coupled with the explosion of information elucidating the human microbiome, open the door to kefir being developed into a probiotic food suitable for clinical applications, for example, as therapy against life-threatening *Clostridium difficile* infections (CDI), with this organism identified as one of the most lethal enteric pathogens (Spinler et al., 2016b). In other words, a systems biology approach (Teusink and Molenaar, 2017) to the kefir microbiota, the fermentation process and the probiotic effects of the beverage (and/or kefir components) would supply all the information needed to enable full exploitation of this already valuable commodity. This is not impossible, since systems biology was applied to *Lb. buchneri*, providing discernment of its genetic equipment, its metabolism, interaction with other microorganisms and gene regulation in response to different environmental conditions (Heinl and Grabherr, 2017). Elucidation of these aspects would be more difficult for a complex microbiota such as kefir, but great strides are made daily toward improvement in and cost reduction of the relevant techniques as well as in the quality and accessibility of bioinformatics tools required to produce data that can be more readily and accurately interpreted (Ferrocino and Cocolin, 2017). Hence, the ideal kefir culture, optimized process parameters, resulting in predictable kefir fermentations and completely defined probiotic properties, are not unrealistic future prospects.

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13

KINETIC CHARACTERISTICS OF ALCOHOL FERMENTATION IN BREWING: STATE OF ART AND CONTROL OF THE FERMENTATION PROCESS

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Symbols

a_s	specific liquid-solid interfacial surface (m^2/m^3)
D	coefficient of molecular diffusion (m^2/s)
D	dilution rate
De	coefficient of effective diffusion (m^2/s)
D_i	diffusion coefficient for the substrate
d_p	particle diameter of the biocatalyst (m)
E_{mx}, E_{mp}	maximum inhibitory concentration of cell growth and ethanol synthesis (g/dm^3)
F	mass feed rate of external substrates in the reactor
FA	higher (fusel) alcohol concentration (mg/dm^3)
$\mathbf{K}$	matrix of constant yield coefficients
$K_{0.5}$	constant
K_{iX}, K_{iP}	constants of inhibition of cell growth and ethanol accumulation (g/dm^3)
K_{LS}, K_{LP}	global mass transfer coefficients for the substrate and the ethanol (h^{-1})
k_s	coefficient of liquid-solid mass transfer (m/s)
K_{SX}, K_{SP}	saturation constants (g/dm^3)
K_{sxi}, K_{spi}	constants of inhibition of cell growth and ethanol synthesis (g/dm^3)
$P(t)$	ethanol concentration (g/dm^3)

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$P_{x\,max}$, $P_{p\,max}$	maximum ethanol concentration at which complete inhibition of cell growth and ethanol accumulation is observed (g/dm^3)
Q	rate of mass outflow from the reactor in gaseous form
$q(t)$	specific product accumulation rate (g/(g h))
$q_{p\,\max}$	maximum specific rate of ethanol accumulation (g/(dm^3 h))
R	radius of the particle of the carrier (m)
r	spherical coordinate (m)
r_f	rate of mass transfer to the surface of the carrier (kg/(m^3 s))
$r_{\max}$	maximum mass transfer rate (kg/(m^3 s))
S	substrate concentration (fermentable extract concentration) (g/dm^3)
$S(t)$	extract (substrate) concentration (g/dm^3)
S_s	substrate concentration at the surface of the carrier (kg/m^3)
VDK	vicinal diketone concentration (mg/dm^3)
V_m	maximum process rate (kg/(m^3 s))
V_m	reaction rate
$X(t)$	biomass concentration (g/dm^3)
Y_{FA}, Y_E, Y_A, Y_{VDK}	yield coefficients of the corresponding metabolite (mg/(g h))
$Y_{X/S}$, $Y_{P/S}$	yield coefficients for biomass and product (ethanol) (g/g (dimensionless))
η	the generalized efficiency factor
η_{ex}	coefficient of efficiency that considers the external diffusion resistances
η_{in}	coefficient taking into account the internal diffusion resistances
$\mu(t)$	specific growth rate (h^{-1})
$\mu_{\max}$	maximum specific growth rate of yeast (h^{-1});
ξ	the vector of concentrations of the components dissolved in the culture broth
φ	vector of reaction kinetics (also called reaction rates)
Φ_i	Thiele modulus
A	aldehyde concentration (mg/dm^3)
E	ester concentration (mg/dm^3)
κ_A, κ_{VDK}	reduction coefficients for aldehydes and vicinal diketones (mg/(g h))

13.1 Introduction

Along with other beer quality forming processes, fermentation is a major process that produces a balanced flavor profile of the beverage. Basically, it is a biochemical process performed by yeast, in which wort is transformed into a finished beer with a specific qualitative and quantitative composition (Boulton, 2006).

As fermentation is known to be the rate-determining process in beer production, the increase in process productivity is associated with changing the fermentation process rate. Increasing the fermentation equipment volume would result in reduction of production costs, but it would lead to higher costs and problems associated with the fermentation process itself. Another approach used in recent years is the production of high-gravity wort, which leads to the production of beer with an increased alcohol content, while reducing the number of production cycles. Although this approach has its economic advantages, it unfortunately affects the quality of the finished beer (Boulton, 2006).

Therefore, the search for other approaches to reduce the duration of the fermentation process and hence the cost of production is key to the current state of technology development. The precise control and management of the fermentation process requires knowledge of fermentation kinetics, not only in order to reduce production costs but also to gain knowledge for the biochemical foundations of the fermentation process (Boulton, 2006).

The purpose of this chapter was to make a critical analysis of the kinetics of the alcohol fermentation process in beer production, both in terms of biochemistry of the process and in terms of its control.

13.2 Microbiology and Biochemistry of Fermentation

13.2.1 Microbiology of Alcohol Fermentation in Brewing

Yeasts are unicellular fungi, most often propagating vegetatively by budding. Yeasts of the *Saccharomyces* genus are most frequently applied in brewing. Two types of yeast are mainly used in beer production: top-fermenting (ale) or bottom-fermenting (lager) yeast. Although in recent years all brewer's yeasts have been referred to the *Saccharomyces cerevisiae* species, lager yeast types are also known as *S. carlsbergensis*, *S. uvarum*, and *S. cerevisiae*. From a classification point of view, the top-fermenting strains are classified entirely as *S. cerevisiae*, but the classification of bottom-fermenting yeast strains is difficult, mainly due to the process of hybridization with yeasts of the *S. monacensis* and *S. bayanus* species. Although in terms of taxonomy yeast classification is precise, the names *S. carlsbergensis* and *S. cerevisiae* for the bottom- and top-fermenting yeasts, respectively, have been retained in practice (Tenge, 2009; Boulton, 2006).

In terms of their basic property to float on the surface or to precipitate at the bottom, at present there are no significant property variations between top-fermenting and bottom-fermenting yeast due to the use of closed fermentation vessels. However, in some breweries where open fermentation systems are still being used, this difference can be seen. Other significant differences between the two yeast types are essential for beer quality: fermentation, substrate consumption rate, temperature tolerance, flocculation characteristics, and the profile of accumulated metabolites. Top-fermenting yeasts ensure the accumulation of more esters, so more fruity notes are felt in the beer, while lager strains provide a cleaner and in some cases even sulfur flavor (Tenge, 2009; Boulton, 2006).

There are a few small differences between the two types of yeasts when it comes to morphology—the shape and size of the cells are similar. Lager strain cells are separated very soon after budding, whereas the cells of the top-fermenting strains remain clingy and continue to bud, which leads to the formation of small clusters (Tenge, 2009; Boulton, 2006).

The most significant differences between the two types of yeasts are in their physiology. The main difference is the consumption of raffinose. The lager yeast enzyme system allows the breakdown of glycosidic bonds between monosaccharides, after which all sugars can be fermented. Top-fermenting strains lack the enzyme melibiase; therefore, there is no breakdown of the glycosidic bond between galactose and glucose, so these sugars cannot be fermented. In this way, only 1/3 of the raffinose is absorbed. It should be noted here that some lager strains also lack galactosidase activity. There is also a difference in the maltotriose utilization, which is better in lager strains. There are differences in the fructose transport systems, too (Tenge, 2009; Boulton, 2006).

The main difference between the two types of yeasts is the temperature range for fermentation. For bottom-fermenting yeasts, the temperature range is 7–15°C, while for top-fermenting yeasts it is between 18°C and 25°C. Data show that lager yeasts grow best at about 28°C but retain their metabolism at low temperatures, making them suitable for cold fermentation and obtaining beer with a better metabolic profile (Tenge, 2009; Boulton, 2006).

13.2.2 Biochemistry of Alcohol Fermentation in Brewing

Beer fermentation biochemistry is complex and many of its aspects remain unclear. Wort is a complex medium and provides a complete growth medium for yeast. As a result of yeast growth and consequently yeast metabolism, not only ethanol and CO_2 are formed during fermentation but also different by-products such as: carbonyl compounds, higher alcohols, esters, organic acids, sulfur-containing compounds, and so on. These secondary metabolites affect beer quality. In fermentation, the choice of the variable yeast strain is often restricted, while fermentation management (including fermentation parameters such as wort composition, temperature, pitching rate, and aeration) has to regulate the conditions so that by-products of yeast growth and metabolism are formed in desired quantities within acceptable time limits (Verbelen and Delvaux, 2009; Shopska et al., 2016). The major metabolic fluxes in the yeast cell are presented in Fig. 13.1 (Tenge, 2009; Boulton, 2006; Bokulich and Bamforth, 2013).

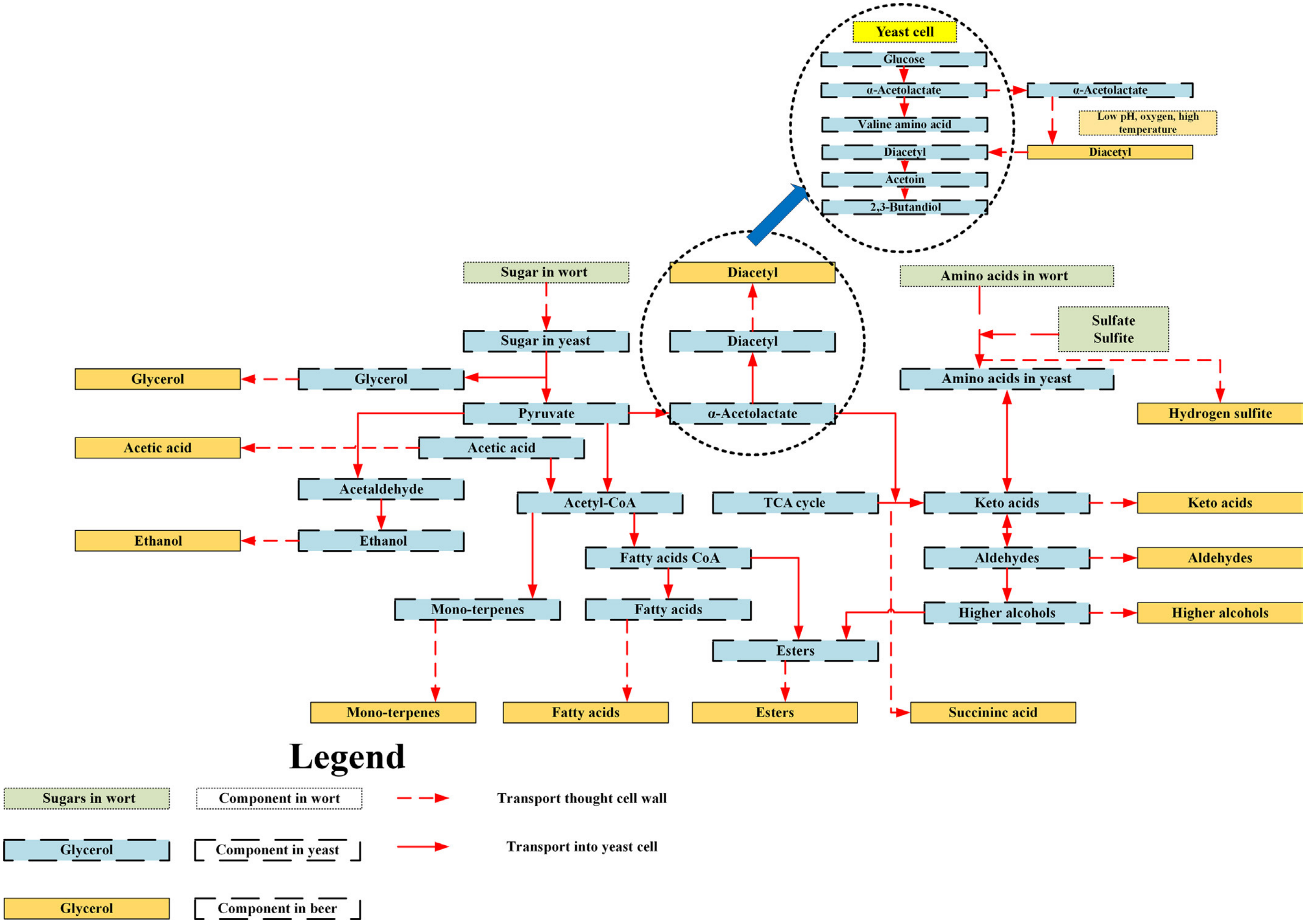


Fig 13.1 Major metabolic fluxes in the yeast cell during alcohol fermentation for the production of beer.

13.2.2.1 Yeast Growth

In a batch fermentation system, yeast growth can be divided roughly into stages or phases: lag phase, logarithmic growth phase, and stationary phase. The lag phase is the time lag, usually 12–24 h, between wort inoculation and the appearance of fermentative activity. However, during the lag phase, important yeast activity occurs. The yeast readjusts to a new environment rich in nutrients. The cells synthesize enzymes needed to utilize nutrients and support growth. Assimilation of oxygen is of particular importance. Molecular oxygen is used by yeast to produce unsaturated fatty acids and sterols, which are essential to cell membrane synthesis. Without sufficient oxygen, yeast growth is restricted and causes abnormal fermentation and flavor changes in beer. Oxygen is consumed rapidly, usually within 6–10 h. Because wort sugars are not being assimilated early in the lag phase, glycogen is essential as an energy source for cell activity. After the lag phase, there is a short transition into the exponential growth phase. In practice, strict logarithmic growth is observed for a short time period only due to the brief period in which sugars are in excess. Cell proliferation occurs by cell budding, in contrast with cell division in other eukaryotes. The individual cells increase in mass and volume to a certain size at which time new buds are formed. The daughter cells (buds) increase in size until, at a critical size, they break from the mother cell, leaving a “bud scar” on the surface of the mother cell. In the logarithmic growth phase, glucose is in excess and the yeast growth rate reaches a maximum. The maximum fermentation rate, which depends on cell population but not on cell growth rate, follows closely in time behind the growth maximum. As the cell population increases and the pools of unsaturated fatty acids and sterols are distributed among the increasing cell population, the growth rate slows. By this time, the cells have begun to assimilate maltose. They gradually shift into the stationary phase as sugar concentration falls. The cells begin to settle or flocculate while the last amounts of the sugars are assimilated at a slow rate. During this period, the glycogen concentration has increased and the cells are preparing for an environment nearly devoid of assimilable carbohydrates. The yeast total dry weight increases throughout fermentation before decreasing slightly at the end of fermentation (Munroe, 2006).

13.2.2.2 Fermentation

Although science has provided a solid understanding of the biochemical processes, fermentation control remains an art of balancing yeast growth and metabolism in a way that the desired flavor compounds are formed within the required process time. The process control parameters such as sufficient nutrient supply, correct inoculation (pitching) rate, optimized dissolved oxygen (DO) addition, and temperature control assist in controlling yeast growth rate and extent. Overall balance in growth (nutrient uptake and by-product release) is required for consistent, brand-specific beer quality (Lodolo et al., 2008).

Carbohydrate Metabolism

The composition of standard brewery wort comprises of approximately 90% of carbohydrates (mainly presented by sucrose, glucose, fructose, maltotriose and maltose, and dextrin material). The qualitative and quantitative profile of wort is mainly dependent on raw materials (malt and adjuncts) and mashing regimes. Yeast utilizes wort sugars in a sequential order after inoculation: glucose, fructose, maltose, and maltotriose. That is due to the catabolite repression of the metabolic pathways for uptake and consumption of wort sugars. Brewers' wort carbohydrates are utilized by yeasts for their growth and by utilizing the energy released during the conversion of sugars into ethanol and are being produced CO_2 and other second metabolites (esters, fusel alcohols, etc.) which give the flavor profile of the final beer (He et al., 2014).

Nitrogen Metabolism

Free amino nitrogen (FAN), are the nitrogenous compounds (amino acids, peptides and ammonium ions) available for consumption by yeast cells. The relative FAN component amounts vary due to the raw materials and process regimes applied and they influence beer flavor. The different wort amino acids lead to different yeast metabolism and in that way they influence the final beer profile. The uptake and utilization of amino acids happens according to a strict order. Group A amino acids are utilized first (arginine, asparagine, aspartate, glutamate, glutamine, lysine, serine, and threonine), followed by the slower disappearance from wort of Group B amino acids (histidine, isoleucine, leucine, methionine, and valine). Group C amino acids (alanine, glycine, phenylalanine, tyrosine, tryptophan as well as ammonia) are only absorbed after the complete removal of group A amino acids. Group D contains only proline, which is poorly utilized. It has no free amino group and therefore cannot be transaminated. The flavor-active compound biosynthesis by the yeast cells is regulated by a complex system and the amino acid composition of wort is an important part of this system (Lodolo et al., 2008; He et al., 2014).

Lipids

Lipids are an important component of the plasma membrane where they are involved in the regulation of the transport of compounds in and out of the cell, regulation of the activities of membrane-bound enzymes, and enhancement of the yeast's ability to resist high ethanol concentrations. *Saccharomyces* yeasts are able to take up fatty acids at low concentrations via facilitated diffusion and at high concentrations via simple diffusion. Free fatty acids are powerful detergents and once taken up by the cell, they are quickly esterified to coenzyme A to reduce their potential for nonspecific enzyme inactivation. The addition of lipids, especially ergosterol and unsaturated long chain fatty acids, has a pronounced effect on yeast growth and metabolism. The addition of the unsaturated fatty acids: oleic, linoleic, and linolenic, has been reported to be a mechanism for the regulation of the concentration of flavor-active compounds in beer (Russell, 2006).

Mineral and Trace Elements

Metals are very important for yeast cell physiology. They are needed to maintain the cell's structural integrity, flocculation, gene expression, cell division, nutrient intake, enzyme activity, and more. The most important metals that influence yeast fermentation are potassium, magnesium, calcium, manganese, iron, copper, and zinc. These are required for yeast metabolism as enzyme cofactors (e.g., manganese) and in yeast respiratory pathways as components of redox pigments (iron and copper). In resume, trace elements and minerals do play an important role in the fermentation and proliferation of yeast cells (Tenge, 2009).

13.2.2.3 Fermentation Products

Main Products

The fermentation process begins with the utilization of glucose in the glycolytic pathway to pyruvate, the major branch point between the fermentation process and the citric acid cycle (Krebs cycle). During fermentation, a net of two ATP molecules are formed as pyruvate is converted via acetaldehyde to ethanol and carbon dioxide (Lodolo et al., 2008). Progressive changes in the ethanol formation rates are observed during batch fermentation. The ethanol formation rate reaches a maximum during the exponential growth phase. Soon after the cessation of yeast growth, the rate of ethanol production declines. It is assumed that the decline in the rates of ethanol production results from the combination of two factors: ethanol toxic effects and nutrient depletion (Boulton and Quain, 2001). The carbon dioxide formed during fermentation can potentially have a negative or a positive impact on the overall brewery performance. The potential negative impact has been observed when yeast exhibits poor fermentation performance. Carbon dioxide is toxic to the yeast cell and efficient nucleation from the fermenting wort is required to minimize impacts on yeast (Kruger et al., 1992; Lodolo et al., 2008).

By-Products

Higher Alcohols The term “higher alcohols” is defined as the amount of compounds with more than two carbon atoms and higher molecular weight and boiling point than ethanol. Also known as fusel alcohols, higher alcohols are the most abundant organoleptic compounds present in beer. Their contribution to beer aroma is usually not very pronounced, due to their relatively high flavor threshold values (10–600 mg/L) (Nedović et al., 2015; Pires and Brányik, 2015). More than 40 higher alcohols in beer have been identified. The most important compounds can be classified into aliphatic [1-propanol, isobutanol (2-methyl-1-propanol), 2-methylbutanol (or active amyl alcohol), and 3-methylbutanol (or isoamyl alcohol)] and aromatic [2-phenylethanol, tyrosol, tryptophol (indole-3-ethanol)] higher alcohols. Aliphatic higher alcohols contribute to the “alcoholic” or “solvent” aroma of beer and produce a warm mouthfeel. The aromatic alcohol 2-phenylethanol has a sweet rose-like aroma and makes a positive contribution to beer aroma. Isoamyl alcohol and 2-phenylethanol can be found around their flavor threshold concentrations in lager beer and can significantly contribute to the flavor of these beers. The latter compound can mask the sweetcorn-like flavor of dimethyl sulfide (DMS). The aromas of tyrosol and tryptophol are undesirable, but they are only present above their thresholds in some top-fermented beers. Besides their own contribution to the overall beer aroma, higher alcohols are precursors of the flavor-intensive esters (Djordjević et al., 2016).

Higher alcohols are produced during fermentation as a result of the Genevois and the Ehrlich pathways. The first one is anabolic (involves carbohydrate synthesis via pyruvate) and the second one is catabolic (synthesis of by-products via amino acid metabolism). The conversion of branched-chain amino acids (e.g., leucine, isoleucine, valine, phenylalanine, and methionine) to higher alcohols via transamination, decarboxylation, and reduction is included in the Ehrlich pathway. During fermentation the listed amino acids are taken up and utilized by yeast cells slowly, in a definite sequential order. The relative contributions of the two metabolic pathways are dependent on the amino acids levels in the fermentation medium. The biosynthetic pathway predominates at low amino acid levels, while the Ehrlich pathway becomes dominant at high levels of amino acids (Nedović et al., 2015). Higher alcohol production is stimulated by conditions that promote yeast cell growth, for example, increased stirring and temperature and high nutrient (oxygen, lipids, amino acids, zinc, etc.) concentrations. The aromatic alcohol synthesis is especially sensitive to changes in the temperature. Logically, the higher alcohol production rate is reduced under conditions that restrict yeast growth, like higher pressure and lower temperature. The types and concentrations of higher alcohols synthesized are significantly influenced by the yeast strain, fermentation conditions, and composition of the wort applied. The synthesis of higher alcohols is also affected by the amino acid composition. The supplementation of wort with valine, isoleucine, and leucine induces isobutanol, amyl alcohol, and isoamyl alcohol production, respectively (Djordjević et al., 2016; Shopska et al., 2016).

Esters Esters are very important flavor compounds in beer since they have a significant effect on the fruity/flowery aromas of beers due to their low flavor threshold despite their presence in low concentrations (Djordjević et al., 2016; Shopska et al., 2016). Esters are mainly formed during the vigorous phase of primary fermentation by condensation of organic acids and alcohols catalyzed by enzymes. Volatile esters in beer can be divided into two major groups: the acetate esters and the medium-chain fatty acid (MCFA) ethyl esters. The former group comprises esters synthesized from acetic acid (acetate) and ethanol or higher alcohol. In the ethyl esters' family, ethanol will form the alcohol radical, and the acid side is an MCFA. Although dozens of different esters can be found in any beer, six of them are of major importance as aromatic constituents: ethyl acetate (solvent-like aroma); isoamyl acetate (banana aroma); isobutyl acetate (fruity aroma); phenyl ethyl acetate (rose and honey aroma); ethyl hexanoate (sweet apple aroma); and ethyl octanoate (sour apple aroma) (Pires and Brányik, 2015).

The availability of acetyl/acyl-CoA and fusel alcohols and the activity of the enzymes are the two factors that determine the ester formation rate. Wort supplementation with MCFAs increases the production of ethyl esters, while higher concentrations of higher alcohols, such as isoamyl alcohol, increase the production rate of the corresponding acetate ester. Ester formation is also influenced by the yeast strain used, temperature, top pressure, stirring, zinc concentration in wort, growth rate, carbon source, and nitrogen concentration. High-gravity brewing significantly increases the concentration of esters, although the type of sugar added to the wort plays an important role. Perhaps the most convenient and selective way to reduce ester production is applying tank overpressure, if necessary in combination with (slightly) lower fermentation temperatures, low wort FAN and glucose levels, and elevated wort aeration or wort lipid concentration. Enhancing ester production is slightly more complicated. If possible, overpressure or wort aeration can be reduced. Otherwise, worts rich in glucose and nitrogen combined with higher fermentation temperatures and lower pitching rates or application of the drauflassen technique may prove helpful (Saerens et al., 2008; Verbelen and Delvaux, 2009; Lei et al., 2012; Hiralal et al., 2014; Shopska et al., 2016; Djordjević et al., 2016).

Carbonyl Compounds About 200 carbonyl compounds are found in beer. Aldehydes and vicinal diketones (VDK) are essential to beer flavor and aroma (Shopska et al., 2016)

- Aldehydes

Aldehydes have different flavor characteristics ranging from "green-leaf-like" to "apple-like" to "citrus-like" to "nutty," depending on the chemical structure, thus contributing to an overall flavor of a number of foods and beverages. Among all aldehydes in alcohol beverages, acetaldehyde is the major component (>90%) giving different flavors depending on its concentration: from a pleasant fruity aroma at low levels to a pungent "green grass-like" odor and "overripe apple" notes at high levels. Acetaldehyde is mostly produced during the yeast active growth phase and accumulated when the carbon dissimilation rate is at its maximum. Some of the acetaldehyde previously excreted is absorbed again and is further reduced to ethanol during fermentation. Thus, its concentration decreases significantly at the end of the fermentation process and then slowly increases over time (Djordjević et al., 2016).

- Vicinal diketones

VDK diacetyl (2,3-butanedione) and 2,3-pentanedione are produced during beer fermentation as by-products of amino acid synthesis (valine and isoleucine, respectively) by yeast of the genus *Saccharomyces*. VDK can significantly influence beer flavor and aroma (Krogerus and Gibson, 2013). Diacetyl is the reason for the "butterscotch"-like aroma and pentanedione—for the "honey"-like aroma (Gamero et al., 2014). However, the flavor threshold of diacetyl is 10 times lower than that of 2,3-pentanedione, making it sensorially more important. Thus, the reduction of the diacetyl concentration below its flavor threshold defines the end of beer maturation for many brewers (Pires and Brányik, 2015). The synthesis and reduction of these components is described in detail in the literature. The diacetyl reduction process by yeast most likely depends on factors like membrane composition, physiological condition, pH and temperature (Krogerus and Gibson, 2013).

13.3 Alcohol Fermentation in Beer Production as an Object of Modeling

The process of alcohol fermentation performed by yeast cells is related to their growth. Placed under appropriate environmental conditions (substrate, pH, temperature), cells begin to proliferate and accumulate biomass. At the same time, the metabolites described in the previous section accumulate in the medium, thus determining beer quality. Since ethyl alcohol is the end product in sugar metabolic pathway, the modeling of the process is facilitated in this case. It is quite different when it comes to modeling the kinetics of the metabolic products (Angelov et al., 2012; Bailey and Ollis, 1986).

Two other processes are closely related to cell growth: the consumption of certain substances from the medium and the release of end products from cellular metabolism in the medium. In general, the prediction of these rates is impossible, and numerous attempts to describe these processes are most often associated with a study of a limited number of typical substrate absorption pathways and the formation of products of the cell's vital activity. The necessary and sufficient accuracy (and therefore complexity) of the mathematical description of the kinetic properties of the systems depends on the complexity of the system itself and suggests an area of use of certain mathematical dependencies (Angelov et al., 2012; Bailey and Ollis, 1986).

The yeast cell population carrying out the process of transforming wort into beer is in constant connection with the medium. Therefore, the fermentation process can be considered as a set of two systems: biological and physicochemical. These two systems are in continuous interconnection, with minor changes in one system being considered as significant changes in the other (Angelov et al., 2012; Bailey and Ollis, 1986).

The physicochemical part of the system determines the nature of the fermentation process. The wort as a nutrient medium is multicomponent and contains all the nutrients necessary for the growth of the yeast population. Wort allows the accumulation of the metabolic products in it thanks to its buffering capacity, which transforms it into the final product, that is, beer. Chemical reactions that are typically associated with the biological phase of the system also occur in the medium. The process of fermentation leads to a change in the pH, which in turn influences the transport processes in the cell population and can lead to an irreversible breach of the beer quality (Angelov et al., 2012; Bailey and Ollis, 1986).

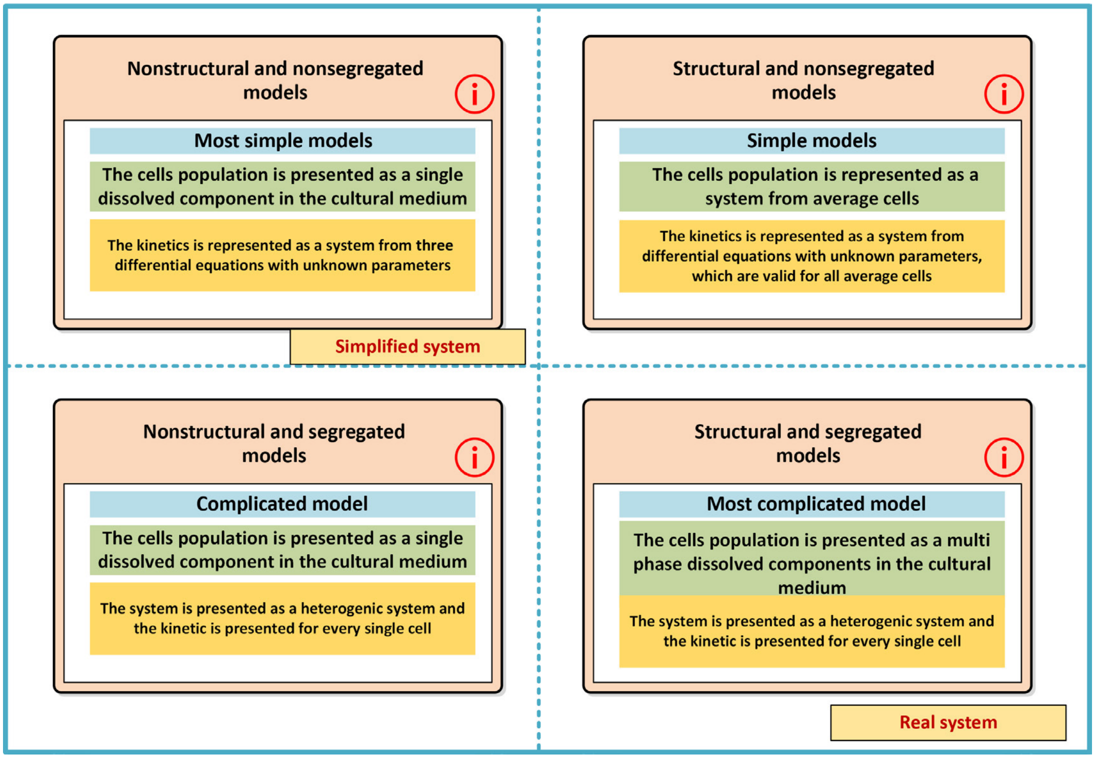
Another important characteristic of the physicochemical part of the system is its heterogeneity, due to the presence of the gas phase, that is, the CO_2 produced by the cells. It promotes the stirring of the fermentation medium, which may, to a certain extent, support the fermentation process, but may also hinder it by the presence of shear forces acting on the cell (Angelov et al., 2012; Bailey and Ollis, 1986).

As far as the most important features of the cell population are concerned, the most important among them is that each cell is a multicomponent system that is even further from being homogeneous at the cellular level. In each cell, multiple reactions are simultaneously carried out by complicated complex regulation systems. Thanks to these systems, the cell can change the rate and even the type of reactions that take place, depending on the conditions and composition of the medium. In the growing cell culture, substantial heterogeneity of the population is observed, that is, at a given time and in a given volume, the cells differ in age (some have just been formed as a result of division, others have reached maturity and still others are in the process of being divided) and, hence, in biochemical activity (Angelov et al., 2012; Bailey and Ollis, 1986).

As can be seen from this brief characterization of the fermentation process, it is obviously difficult to create models (mathematical, kinetic) to describe all the elements of the fermentation process. For this reason, the fermentation process should be considered with a number of simplifications that only refer to the main products of the metabolism of the yeast population (Angelov et al., 2012; Bailey and Ollis, 1986).

In their works, Bailey and Ollis (1986) and Pirt (1975) have suggested several ways of simplifying the dependencies, which in turn lead to the creation of several groups of models that could describe each fermentation process, including alcohol fermentation for beer production (Fig. 13.2).

According to this scheme, the approaches to the analysis of microbiological systems are classified according to the number of


Nonstructural and nonsegregated models
Most simple models
Structural and nonsegregated models
Simple models
Simplified system
Nonstructural and segregated models
Complicated model
Structural and segregated models
Most complicated model
Real system

The cells population is presented as a single dissolved component in the cultural medium

The kinetics is represented as a system from three differential equations with unknown parameters

The cells population is represented as a system from average cells

The kinetics is represented as a system from differential equations with unknown parameters, which are valid for all average cells

The cells population is presented as a single dissolved component in the cultural medium

The system is presented as a heterogenic system and the kinetic is presented for every single cell

The cells population is presented as a multi phase dissolved components in the cultural medium

The system is presented as a heterogenic system and the kinetic is presented for every single cell

Fig. 13.2 Systemic approaches to describe the kinetics of the fermentation process according to Bailey and Ollis (1986) and Pirt (1975).

components used to describe the cells as well as whether the population is heterogeneous or consists of average-sized cell. Multicomponent models are called structural, while one-component models are nonstructural (Angelov et al., 2012; Bailey and Ollis, 1986).

The allowances for these models are different, but they can be reduced to a few key points (Angelov et al., 2012; Bailey and Ollis, 1986):

- It is assumed that one or several components in the nutrient medium determine the course of the process, and the other components are in surplus and do not affect the overall process rate. In our case, wort sugars have the most significant influence on the rate of alcohol fermentation.

This assumption is true if we look at the process only in terms of ethanol accumulation. However, this is not true if we look at the process in terms of synthesis and reduction of VDK. It is well known that if there are no Group A amino acids present in the medium, the cells will synthesize them, which will lead to increased levels of VDK in the fermentation process, which in turn will prolong the process of brewing and maturation of beer.

- Another assumption is that some of the accumulated metabolites lead to inhibition of cell growth. In the case at hand, this is ethanol, which, combined with low fermentation temperatures, can cause the alcohol fermentation process to cease.
- It is assumed that the other parameters of the fermentation process are not significantly influenced and/or can be controlled.

However, in some cases, an adequate description of growth kinetics may require the model to account for the multi-componency of the system and some of the environmental parameters.

What has been said so far shows that the process of alcohol fermentation in beer production is complex both for description and control. This complexity is determined not so much by any physical difficulty, but rather by the fact that we ultimately receive a product to be consumed and whose flavor profile is recognizable.

13.4 Models for Description of the Fermentation Process in Classical Fermentation Systems (Free Cell Systems)

13.4.1 Models Describing the Primary Metabolism of the Yeast Population

To describe primary metabolism, we will use the changes in the concentrations of the substrate, the biomass and the ethanol in the fermenting wort. The typical dynamics of the fermentation process is presented in Fig. 13.3 (data are obtained by fermentation of laboratory

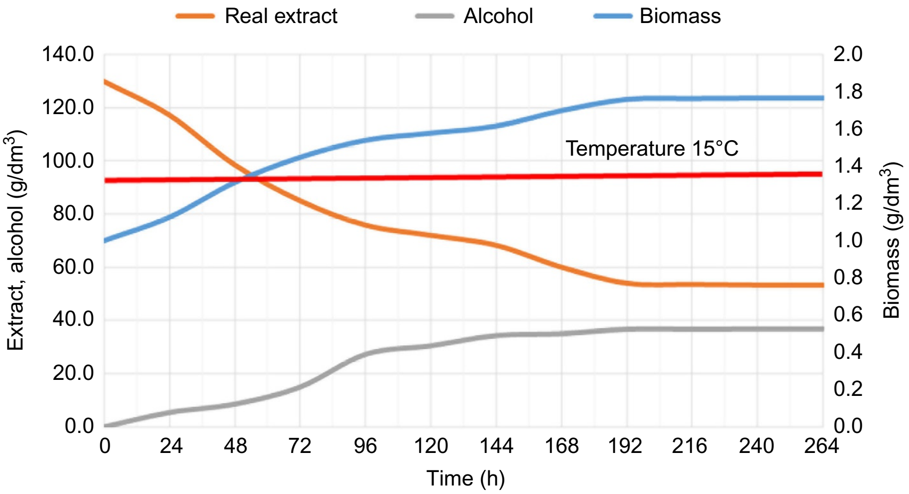


Fig. 13.3 Typical dynamics of the fermentation process (data from laboratory fermentation processes conducted in the laboratories of the Department of Wine and Brewing).

wort with initial extract of 13% and fermentation with yeast strain *S. cerevisiae* W34/70).

Primary metabolism can be summed up with a system of three differential equations (13.1) that reflect the three basic parameters of the biological system: biomass accumulation, ethanol accumulation, and substrate absorption. In general, the kinetic parameters of the system μ, q, $Y_{X/S}$, and $Y_{P/S}$ should be considered both as a function of the fermentation temperature but also of the time variables. Since in general beer fermentation temperature changes over time (in the process maturation it may be lower or higher than the main fermentation temperature), this should lead to a difference in kinetic parameters for both fermentation stages, which would create some difficulties in the modeling process (Parcunev et al., 2012).

$$\left|\begin{array}{l} \dfrac{dX}{dt}=\mu(t,T)X(t,T) \\ \dfrac{dP}{dt}=q(t,T)X(t,T) \\ \dfrac{dS}{dt}=-\dfrac{1}{Y_{x/s}}\dfrac{dX}{dt}-\dfrac{1}{Y_{p/s}}\dfrac{dP}{dt} \end{array}\right. \tag{13.1}$$

In this case, there are different approaches that are used by different authors for simplification, that is, excluding the temperature as a parameter or including the temperature in a certain form in the kinetic parameters. In brewing practice, a variant has been applied whereby the fermentation temperature is constant over the entire period and therefore leads to simplification of dependencies. In this situation, we can discuss several cases of description of the primary cell metabolism.

In their work, Parcunev et al. (2012), Vassilev et al. (2013), and Shopska et al. (2016), discuss the modeling of the alcohol fermentation process, which is carried out for 10 days at a constant fermentation temperature of 15°C. In this case, the differential equation system (13.1) is simplified (13.2). To describe the kinetic parameters, the authors use Monod dependence. Although the Monod equation is the simplest dependence, it is appropriate for describing cell growth in the case of longer fermentation because it averages what is happening in the yeast population.

$$\left|\begin{array}{l}\dfrac{dX}{dt}=\mu X\\[2mm] \dfrac{dP}{dt}=qX\\[2mm] \dfrac{dS}{dt}=-\dfrac{1}{Y_{x/s}}\dfrac{dX}{dt}-\dfrac{1}{Y_{p/s}}\dfrac{dP}{dt}\end{array}\right. \qquad (13.2)$$

$$\mu=\mu_{\max}\frac{S}{K_{sx}+S};\quad q=q_{p\max}\frac{S}{K_{sp}+S}$$

This simpler approach of using a nonstructural model and the Monod equation enables the obtaining of data on the kinetics of the fermentation process rapidly and easily, and the data would then be interpreted and/or implemented in a process management algorithm. Data from the identified kinetics of the fermentation process presented in Fig. 13.3 are shown in Table 13.1. Table 13.3 also presents data on the kinetics of a process with immobilized cells and the secondary metabolism of the two fermentations which are discussed later. There are some difficulties in using the equation system (13.2), complemented by the Monod model. These difficulties are related to the fact that the model does not describe well the passage of cells from the exponential to the stationary growth phase. This in turn is reflected in both the accuracy of the model and in the description of the changes in the extract and alcohol concentrations (Fig. 13.4). Data from the works of Parcunev et al. (2012), Vassilev et al. (2013), Shopska et al. (2016) show that the use of the Monod model leads to an enhanced modeling error (the error ranges from 0.3 to 0.5), which reflects in the correlation coefficient that varies around $R^2=75\%$–80%. These data are also confirmed by our other observations, one of these observations being reflected in Fig. 13.4. The data quoted in Parcunev et al. (2012), Vassilev et al. (2013), Shopska et al. (2016) show that Monod model is difficult to adapt to the dampening fermentation process, especially in the maturation phase. This in turn

Table 13.1 Kinetic Parameters in the Monod Model for Primary and Secondary Metabolism at a Constant Fermentation Temperature of 15°C

	Primary Metabolism							
Fermentation type	$\mu_{\max}$	K_{sx}	$q_{p\max}$	K_{sp}	$Y_{X/S}$	$Y_{P/S}$	$\eta\mu$	η_q
Free cells	0.0222	237	1.25	503	0.47	0.43	5.40	8.66
Immobilized cells	0.012	39.15	10.83	323.15	0.0154	1.25		
Secondary Metabolism							**Mass Transfer**	
–	Y_A	k_A	Y_E	Y_{FA}	Y_{VDK}	K_{VDK}	K_{LS}	K_{LP}
Free cells	0.1177	0.0136	0.1732	0.0733	0.0065	0.0285	–	–
Immobilized cells	0.0331	0.005	0.21	0.059	0.0042	0.0125	0.0052	0.01
–	η_A	η_{KA}	η_E	η_{FA}	η_{VDK}	η_{KVDK}	–	–
–	0.281	0.368	1.212	0.805	0.646	0.439	–	–

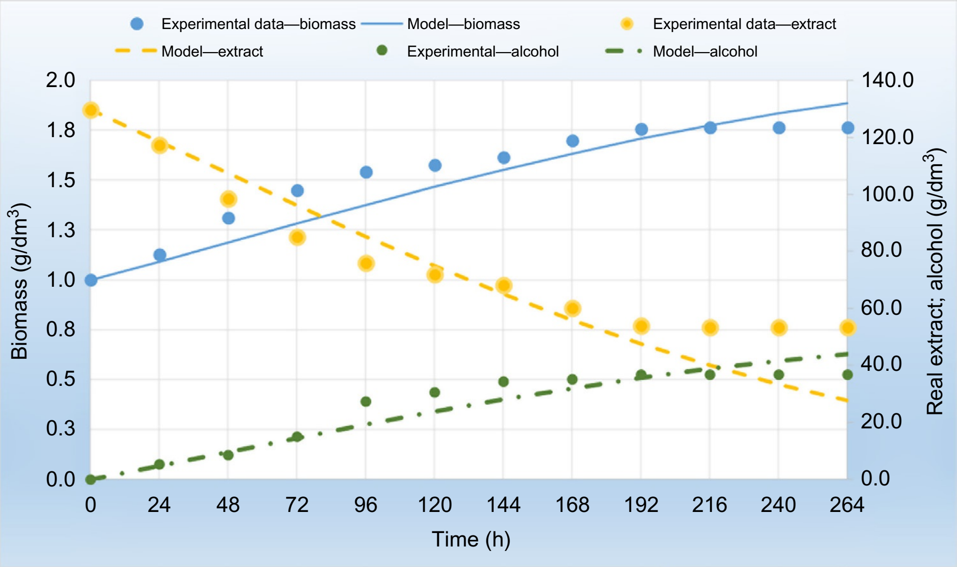


Fig. 13.4 Comparison of experimental fermentation data with the Monod model (data from laboratory fermentation processes conducted in the laboratories of the Department of Wine and Brewing).

leads to deviations in the description of the extract and the alcohol concentrations. The correlation coefficient does not exceed 80% in these parameters either (Fig. 13.4).

The observed low correlation between the experimental data and the Monod model is due to some processes in the biological system that Monod proposed equation does not take into account. They are substrate inhibition, product inhibition, and the order of sugar utilization in the system. The inclusion of these three biological processes in the kinetic model in one form or another improves the accuracy of the description.

Various model types are used in the scientific field for the description of the substrate and product inhibition processes. They can be included in the model, both individually and in a combination. The latter would complicate the model, but in most cases it increases its accuracy.

It is well known that the alcohol fermentation process, whatever the product is, beer, wine, or ethanol, is a product-inhibited process. This means that the accumulation of the major metabolite reduces the rate of cell growth, which also affects the overall process dynamics. This may affect the models in different ways.

Parcunev et al. (2012) and Shopska et al. (2016) suggest two models for describing the impact of product inhibition. The first model (13.3) is the use of the Aiba equation, which suggests an exponential dependence of inhibition depending on the concentration of ethanol in the medium.

$$\left|\begin{array}{l} \dfrac{dX}{dt} = \mu X \\ \dfrac{dP}{dt} = qX \\ \dfrac{dS}{dt} = -\dfrac{1}{Y_{x/s}}\dfrac{dX}{dt} - \dfrac{1}{Y_{p/s}}\dfrac{dP}{dt} \end{array}\right. \tag{13.3}$$

$$\mu = \mu_{\max}\frac{S}{K_{sx}+S}e^{(-K_{ix}P)};\quad q = q_{p\max}\frac{S}{K_{sp}+S}\mathrm{e}^{(-K_{ip}P)}$$

Product inhibition of the process is most pronounced at its end when the combination of high ethanol concentration and low fermentation temperature starts leading to limitations in cell growth. The results obtained by Parcunev et al. (2012) referring to fermentation carried out with free and immobilized bottom-fermenting yeast are supplemented by results from fermentation with two other starting wort extracts: 15% and 17%. Modeling of the yeast fermentation process under the same conditions was conducted as well (Parcunev et al., 2012; Kostov, 2015). From the research carried out, the following conclusions can be drawn:

- The supplementation of the Monod equation with a parameter that reflects product inhibition does not substantially improve the model accuracy. The error between the experimental and the model data is comparable and in some cases even higher than that of the previous system of differential equations. This leads to a similar correlation between model and experimental data, ranging from 80% to 85%.

- The data on the inhibition constant values in the Aiba equation show that product inhibition affects cell growth to a greater extent and, to a lesser extent, ethanol accumulation. There was no correlation between the initial wort extract and the degree of product inhibition (Fig. 13.5).
- From the data quoted in Parcunev et al. (2012) and Kostov (2015), it can be concluded that in the yeast strain and fermentation conditions used, product inhibition was relatively low.

The latter statement of relatively weak inhibition cannot be explained with the data from the Aiba model since the obtained kinetic constant in it is in the exponent, which enhances its influence. Ghose and Thyagi's dependence (13.4) can be used to demonstrate the degree of inhibition of the product. A key parameter in this model is the maximum product concentration (in this case, ethanol), which will result in complete inhibition of the cell growth and the accumulation of the target product.

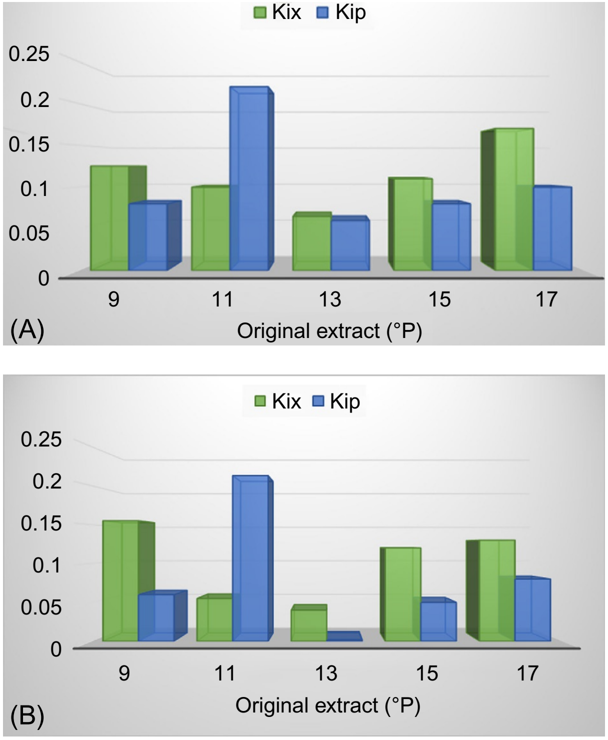


Fig. 13.5 Inhibition constants in the Aiba model for different types of fermentation processes. (A) *S. carlsbergensis* S-23; (B) *S. cerevisiae* S-33.

$$\left|\begin{aligned} &\frac{dX}{dt} = \mu X \\ &\frac{dP}{dt} = qX \\ &\frac{dS}{dt} = -\frac{1}{Y_{x/s}}\frac{dX}{dt} - \frac{1}{Y_{p/s}}\frac{dP}{dt} \end{aligned}\right.$$

$$\mu = \mu_{\max}\left(1 - \frac{P}{P_{x\max}}\right); \quad q = q_{p\max}\left(1 - \frac{P}{P_{p\max}}\right) \tag{13.4}$$

The Ghose and Thyagi model is suitable for describing processes that are highly susceptible to product inhibition. Applied to beer fermentation data, it indicates that full inhibition of cell growth and ethanol synthesis will occur at ethanol concentrations in beer between 5 and 10 times as high as the real values that can be detected in beer, even with the use of high-efficiency wort, in which it is assumed that more ethanol will be accumulated due to the higher wort extract. The cited data proves the presence of a slight inhibition of the process, which is also found in the application of the Aiba equation.

Another possible approach to introducing product inhibition is a modification of the Monod model (13.5). This approach increases the model precision in terms of experimental data, but makes it difficult for interpretation. Petelkov et al. (2016) used this model to describe a fermentation process with top-fermenting yeast, showing that the accuracy of the model ranged from 85% to 90%. The difficulty in interpreting the data is that the inhibition constants are found in the Monod equation denominator, and the product concentration is elevated to a square. This increases accuracy and it suggests that even minor deviations in the product concentration would lead to severe inhibition. Ethanol accumulation is poor and yeasts are adapted to the fermentation during alcohol fermentation in brewing.

$$\left|\begin{aligned} &\frac{dX}{dt} = \mu X \\ &\frac{dP}{dt} = qX \\ &\frac{dS}{dt} = -\frac{1}{Y_{x/s}}\frac{dX}{dt} - \frac{1}{Y_{p/s}}\frac{dP}{dt} \end{aligned}\right. \tag{13.5}$$

$$\mu = \mu_{\max}\frac{S}{K_{sx} + S + \dfrac{P^2}{K_{SXi}}}; \quad q = q_{p\max}\frac{S}{K_{sp} + S + \dfrac{P^2}{K_{SPi}}}$$

The models discussed so far comment on the impact of product inhibition. From the review of the biochemistry of the alcohol fermentation process, it appears that the fermentation process is substrate inhibited by the glucose concentration, and the fermentation of the remaining sugars begins only after the almost complete depletion of the glucose. This is when the absorption of fructose, maltose, and maltotriose from the wort begins. This process should be reflected in the kinetic model in a certain way. Again, there are different approaches, the accuracy of which depends on the assumptions made.

To describe the substrate inhibition process, Ramirez and Maciejowski (2007) suggest a description of the kinetics of the fermentation process through a number of equations that are reflected in Table 13.2.

The main features of this model are as follows:

- Glucose utilization follows Monod-based kinetics. According to the biochemistry of the process, maltose utilization is inhibited by the presence of glucose in the wort, which is reflected by the member $\frac{K_G^{'}}{K_G^{'}+G}$. This means that the inhibition of maltose utilization is described by an equation similar to that of Monod-Jerusalemski for substrate-product inhibition of the fermentation process (Gaponov, 1981). The maltotriose utilization is doubly inhibited by the presence of both glucose and maltose in the medium and is reflected by $\frac{K_G^{'}}{K_G^{'}+G}\frac{K_M^{''}}{K_M^{''}+M}$. The absorption of maltose and maltotriose is described with Monod-based kinetics and is inhibited by the concentration of glucose and maltose (for maltotriose) in wort.
- The relation of the kinetic parameters to the fermentation temperature is given by an equation similar to that of the Arrhenius equation for chemical kinetics. This part of kinetics is valid for all three types of sugars in wort. These dependencies also describe the saturation and inhibition constants in the sugar utilization equations.
- The total biomass accumulation rate is described with Monod-based kinetics, with the maximum specific growth rate of the microbial population being the average of the biomass growth rates determined by the utilization of the three major types of sugars in the medium: glucose, maltose, and maltotriose. This is reflected in the yield (economic) coefficients Y_{XG}, Y_{XM}, and Y_{XN}. The volume of the inoculum at the beginning of the fermentation process also influences the growth rate.
- The ethanol accumulation is determined by the concentrations of the three types of sugars in the wort multiplied by the yield coefficients Y_{EG}, Y_{EM}, and Y_{EN}.

Table 13.2 Kinetic Equations Proposed by Ramirez and Maciejowski (2007)

BIOMASS GROWTH AND UTILIZATION OF THE SUBSTRATE		
Glucose (G)	$\frac{dG}{dt} = -\mu_1 X$	Influence of the temperature on the kinetic parameters $\mu_i = \mu_{i0} e^{\left(\frac{-E_{Ki}}{RT}\right)}, \quad i = G, M, N$ $K_i = K_{i0} e^{\left(\frac{-E_{Ki}}{RT}\right)}, \quad i = G, M, N$ $K'_i = K'_{i0} e^{\left(\frac{-E'_{Ki}}{RT}\right)}, \quad i = G, M, N$
Maltose (M)	$\frac{dM}{dt} = -\mu_2 X$	
Maltotriose (N)	$\frac{dN}{dt} = -\mu_3 X$	
$\mu_1 = \mu_{\max G} \frac{G}{K_G + G}$ $\mu_2 = \mu_{\max M} \frac{M}{K_M + M} \frac{K'_G}{K'_G + G}$ $\mu_3 = \mu_{\max N} \frac{N}{K_N + N} \frac{K'_G}{K'_G + G} \frac{K''_M}{K''_M + M}$		Total rate of biomass accumulation $\frac{dX}{dt} = \mu_X X$ $\mu_X = \left(Y_{XG}\mu_1 + Y_{XM}\mu_2 + Y_{XM}\mu_3\right) \frac{K_X}{K_X + \left(X - X_0\right)^2}$

Ethanol accumulation

$$E = E_0 + Y_{EG}(G_0 + G) + Y_{EM}(M_0 - M) + Y_{EN}(N_0 - N)$$

Changes in the temperature during fermentation

$$\frac{dT}{dt} = \frac{1}{\rho cp}\left(\Delta H_{FG}\frac{dG}{dt} + \Delta H_{FM}\frac{dM}{dt} + \Delta H_{FN}\frac{dN}{dt} + u(T - Tc \right)$$

Utilization of amino acids from the wort

Leucine (L)

$$\frac{dL}{dt} = -Y_{LX}\frac{dX}{dt}\frac{L}{K_L + L}D$$

Isoleucin (I)

$$\frac{dI}{dt} = -Y_{IX}\frac{dX}{dt}\frac{I}{K_I + I}D$$

Valine (V)

$$\frac{dV}{dt} = -Y_{VX}\frac{dX}{dt}\frac{V}{K_V + V}D$$

$$D = 1 - e^{\frac{t}{\tau_D}}$$

- The absorption of some of the main amino acids in wort is also described with Monod-based kinetics, but the model suggests some delay in their absorption due to the fact that they are considered group B amino acids, whose digestion begins only after the amount of group A amino acids finishes. The delay is exponentially determined by the coefficient D.

The model proposed by Ramirez and Maciejowski (2007) can be described as a structural nonsegregated model as it describes the fermentation process in greater detail. Based on the biochemistry of the process, it makes it possible to obtain data on the absorption of the various sugars in the environment and to develop more precise control of the fermentation process through these data. The main difficulty that may be encountered in using this model is the fact that it is necessary to know the wort composition with regard to sugars and amino acids every time, which naturally makes it difficult to work with it.

The values obtained by the model suggested by Ramirez and Maciejowski (2007) are also interesting. Some of them are presented in Table 13.3. The data show that glucose utilization determines the fermentation process rate. Absorption of maltose and maltotriose is two and three times, respectively, as slow as that of glucose. The threefold increase in the biomass yield coefficient by maltotriose unit Y_{XN} compared to Y_{XG}, which corresponds to the fact that maltotriose consists of three units of glucose, is also interesting.

Table 13.3 Values of Some of the Kinetic Parameters Identified by Ramirez and Maciejowski (2007)

Parameter	Value
$\ln\mu_{G0}$, $\ln(h^{-1})$	35.77
$\ln\mu_{M0}$, $\ln(h^{-1})$	16.4
$\ln\mu_{N0}$, $\ln(h^{-1})$	10.59
$\ln K_{G0}$, ln(gmol/cu m)	−121.3
$\ln K_{M0}$, ln(gmol/cu m)	−19.5
$\ln K_{N0}$, ln(gmol/cu m)	−26.78
Y_{XG}	0.134
Y_{XM}	0.268
Y_{XN}	0.402
Y_{LX}	0.0832
Y_{IX}	0.0363
Y_{VX}	0.0273
τ_D, h	24.54

The parameters presented in Ramirez and Maciejowski (2007), and supplemented by Gee and Ramirez (1988) and Gee and Ramirez (1994) show that the proposed model has a high degree of compliance with experimental results. Unfortunately, some of the parameters in it are not identified, which indicates that such complex models present a difficulty with their computer processing. However, this model is useful due to the fact that it is possible to confirm in a digital form the described biochemical processes that occur in the yeast population during fermentation. For example, the cited data in Table 13.3 show that the consumption of group B amino acids begins about 24 h after the beginning of fermentation, and the economic coefficients, that in this case can be considered as rate constants, are close.

The simultaneous influence of product and substrate inhibition can also be achieved by using a simpler nonstructural model (13.6).

This pattern suggests the presence of linear product inhibition as determined by the member $\left(1-\dfrac{P}{E_{mx}}\right)$ and the presence of quadratic substrate inhibition. This model is used by Petelkov et al. (2016) to describe free cell fermentation processes at constant temperature. The results obtained with it show that its accuracy is very high: the correlation coefficient in the case study is over 90%. The evaluation of its parameters indicates that complete inhibition occurs at ethanol concentrations between three and five times as high as those achievable in beer. Substrate inhibition is of basic importance due to the order of sugar utilization.

$$\left|\begin{aligned}&\frac{dX}{dt}=\mu X\\&\frac{dP}{dt}=qX\\&\frac{dS}{dt}=-\frac{1}{Y_{x/s}}\frac{dX}{dt}-\frac{1}{Y_{p/s}}\frac{dP}{dt}\end{aligned}\right. \tag{13.6}$$

$$\mu=\mu_{\max}\left(1-\frac{P}{E_{mx}}\right)\frac{S}{K_{sx}+S+\dfrac{S^2}{K_{SXi}}};\quad q=q_{p\max}\left(1-\frac{P}{E_{mp}}\right)\frac{S}{K_{sp}+S+\dfrac{S^2}{K_{SPi}}}$$

The last model that we discuss in this part was suggested in the work of de Andres-Toro et al. (1998) (Table 13.4). The model implies Monod-based kinetics of biomass growth and can be considered as a nonstructural segregated model for the following reasons:

- Biomass is divided into three types of cells: lag (X_{lag}), active (X_{act}), and dead (X_{dea}) cells, and the relationship between the three cell types is given by the relations presented in Fig. 13.6. In this case,

Table 13.4 A Mathematical Model for Describing the Fermentation Process Kinetics Proposed by de Andres-Toro et al. (1998)

CELL GROWTH

- Inoculum

$$X_{act}(0) + X_{lag}(0) = 0.5X_{inc}(0) \quad t < t_{lag}$$

- Biomass immediately after inoculation

$$X_{sus}(t) = X_{act}(t) + X_{lag}(t) + X_{dea}(t)$$

- Decrease of suspended cells because of dead cells settling at the bottom with rate μ_{SD}

$$\frac{dX_{sus}(t)}{dt} = -\mu_{SD} X_{DT}(t) = -\mu_{SD}\left[X_{sus}(t) - 0.5X_{inc}\right] \; t < t_{lag}$$

$$\mu_{SD} = \mu_{SD} \frac{0.5C_{S0}}{0.5C_{S0} + C_e(t)}$$

- Activation of lag-phase cells with rate μ_L

$$\frac{dX_{act}(t)}{dt} = \mu_{lag} X_{lag}(t) = \mu_L\left(0.5X_{inc} - X_{act}(t)\right) t < t_{lag}$$

- Biomass growth during the fermentation process

$$\frac{dX_{act}(t)}{dt} = \mu_X X_{act}(t) - \mu_{DT} X_{act}(t) + \mu_L X_{lag}(t) t > t_{lag}$$

$$\mu_X = \mu_{X0} \frac{C_s(t)}{k_X + C_e(t)}$$

Substrate utilization

$$\frac{dC_s(t)}{dt} = -\mu_S X_{act}(t)$$

Ethanol accumulation

$$\frac{dC_e(t)}{dt} = f \mu_e X_{act}(t)$$

$$f = 1 - \frac{C_e(t)}{0.5C_{S0}}$$

$$\mu_e = \mu_{e0} \frac{C_S(t)}{ke + C_S(t)}$$

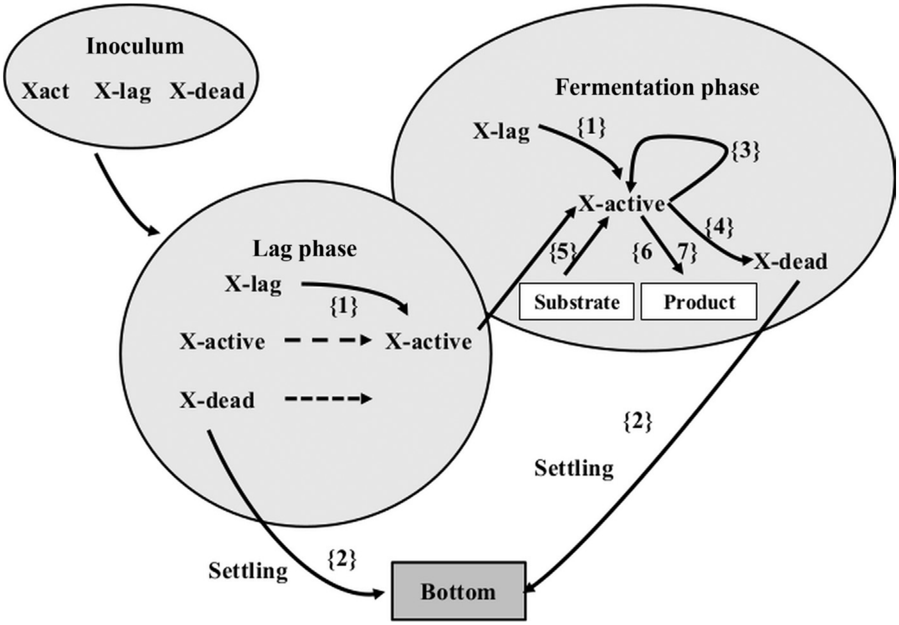


Fig. 13.6 Principal scheme of kinetics of the fermentation process in beer production, according to de Andres-Toro et al. (1998) (via www.sciencedirect.com).

fermentation is divided into two phases: a lag phase (in which no fermentation is observed) and a fermentation phase. The lag phase of the process begins immediately after inoculation of the wort and consists of only two steps: death of the cells and activation of the cells. The lag phase ends when 80% of the inoculum turns into active cells. This is when the fermentation phase begins. The time for the lag phase is assigned by the authors as t_{lag}.

- The model requires knowledge of the proportions of the individual cells in the inoculum: dead cells, lag-phase cells and active cells. Immediately after inoculation, the cells enter a suspended state, and the dead inoculum cells settle at the bottom (reaction 13.2 from Fig. 13.6) at rate μ_{SD}. This rate is proportional to the wort extract C_{S0} and the amount of carbon dioxide (which can be determined from the concentration of ethanol C_e).
- Cells pass from lag state to active state by the end of the lag phase at an activation rate μ_L.
- The biomass accumulation during fermentation is described with the Monod-based kinetics of the growth characteristic μ_X. The change in biomass concentration during fermentation is proportional to the current biomass growth, cell death, and activation of lag-phase cells. Here, in the equation for the specific growth rate μ_X, the saturation constant is replaced by the ethanol concentration, which results in the consideration of the product inhibition on the growth.

- The substrate utilization and the ethanol accumulation are described with Monod-based equations, their variation being proportional to the concentration of active cells during the actual fermentation. Data by the authors show that ethanol accumulation is not monotonic and with the increase in ethanol concentration, there is inhibition, which should be reflected by a proportionality coefficient ***f***. It is proportional to the concentration of ethanol in beer and the maximum concentration of the product that can be achieved using the respective wort.

The model proposed by de Andres-Toro et al. (1998) is used to describe fermentation processes in real conditions, and according to the authors it gives good results and allows the kinetic parameters to be described with equations in which the fermentation temperature is present. This, in turn, allows optimization of the fermentation process.

13.4.2 Modeling of the Secondary Metabolism of the Yeast Population

In contrast to primary metabolism, where different variations in the models that describe it are possible, in secondary metabolism, cell-associated relationships are used. Only in case of VDK some differences can be observed, mainly due to the presence of a chemical phase in their reduction. The basis of the secondary metabolism models is the biochemistry of the process, and their form is presented in Table 13.5.

In some cases, models for specific representatives of a given group of metabolites (ethyl acetate, ethyl caproate, etc.) can be found in the literature, but the tendency for their accumulation to be associated with biomass growth remains. The accuracy of the secondary metabolite models depends largely on the accuracy of the chosen model for describing the growth rate of the yeast cells, but there are also some features related to the biochemistry of the process. These peculiarities can be summarized as follows:

- *Higher alcohol synthesis*: higher alcohols are mainly synthesized (over 90%) during the main fermentation, therefore the biomass growth rate in this period is essential. The fermentation temperature is the main factor influencing the yield coefficient Y_{FA};
- *Ester synthesis*: 60% of the esters are synthesized during the exponential phase and the remaining 40% during the stationary phase of biomass growth. Their synthesis is delayed compared to that of higher alcohols, since the latter are precursors to ester synthesis. This delay, however, is not commented by the model; the model is associated with biomass growth and its accuracy depends on the accuracy of the cell growth description;
- *Aldehyde synthesis and reduction*: the formation of aldehydes during fermentation is associated with cell growth and the biosynthesis

Table 13.5 Mathematical Model for the Description of Secondary Metabolites, According to Ramirez and Maciejowski (2007)

Higher alcohol synthesis (*FA*)

$$\frac{dFA}{dt} = Y_{FA} \times \mu \times X(t)$$

Ester synthesis (*E*)

$$\frac{dE}{dt} = Y_E \times \mu \times X(t)$$

Aldehyde synthesis and reduction (*A*)

$$\frac{dA}{dt} = Y_A \times \mu \times X(t) - k_A \times A \times X$$

Vicinal diketones (*VDK*)

$$\frac{dVDK}{dt} = Y_{VDK} \times \mu(t,T) \times X(t,T) - k_{X,VDK} \times VDK(t,T) \times X(t,T)$$

of higher alcohols from yeast oxo acids. They increase during the main fermentation and decrease during maturation. Their reduction is entirely related to the already formed biomass, so there is also a second member in the equation, which is a function of the already synthesized biomass.

- *Vicinal diketones*: diacetyl and 2,3-pentanedione are synthesized during cell growth, so their increase is proportional to cell growth. In contrast to aldehydes, chemical reduction is possible in VDK, the rate of which depends on the fermentation temperature. At low fermentation temperatures, this process is very slow and may not be taken into account in the model for these metabolites. Reduction of diacetyl and 2,3-pentanedione is due to their consumption by yeasts and their transformation to the corresponding alcohol: 2,3-butanediol and 2,3-pentanediol. The reduction rate is proportional to the concentration of biomass already accumulated and the current concentration of VDK.

Details of the parameters identified for these models are presented in Table 13.1. The data make it possible to compare different fermentation regimes with respect to the yields of the relevant metabolites. The accuracy of these models is entirely dependent on the chosen model for describing the primary metabolism. Furthermore, the accuracy of the secondary metabolism models depends on the fermentation temperature. We can summarize that yield coefficients can also be regarded as rate constants as they depend on the temperature.

13.5 Models for Description of the Fermentation Process in Innovation Fermentation Systems (Systems With Immobilized Cells)

13.5.1 Common Features of Immobilized Cell Systems and the Diffusion Resistances in Them

Immobilized cells are physically limited or localized in a specific space while preserving their catalytic activity, and if possible, and even necessary, viability, and which can be used repeatedly and continuously (Godia et al., 1987).

The use of immobilized cell systems offers a number of advantages (Boulton and Quain, 2001; Hayes et al., 1991; Pilkington et al., 1998): increased cell concentration in the reactor operating volume and increased reaction rate; smaller bioreactor sizes, and in continuous processes—lower reaction times; easy separation and regeneration of biomass; possibility for use in both batch and continuous alcohol fermentation. Immobilization is characterized by the following disadvantages (Mensour et al., 1996): limited mass exchange due to the presence of diffusion resistances; possibility of destroying the matrix at a high rate of the fermentation process and/or the formation of gaseous metabolites; changes in the physiological state of the immobilized culture as well as in the growth rate and the overall stoichiometry of the reactions in the immobilized system.

The basic immobilization methods are presented in Fig. 13.7. They can be divided into four main groups (Kourkoutas et al., 2004): binding of cells to the surface of preformed and insoluble carriers (Fig. 13.7A); physical inclusion in porous matrices (Fig. 13.7B); formation of cell aggregates using natural flocculation or crosslinking with chemical agents (Fig. 13.7C); immobilization behind membranes (Fig. 13.7D).

The choice of suitable facilities and conditions for carrying out the various processes with immobilized cells requires both a detailed knowledge of the biochemical and the microbiological features, but also knowledge of diffusion resistances in the system as they determine its behavior to a large extent. The description of the mass transfer limiting constraints is one of the most complex issues of immobilized enzyme and cell processes. Different dependencies, mostly adapted

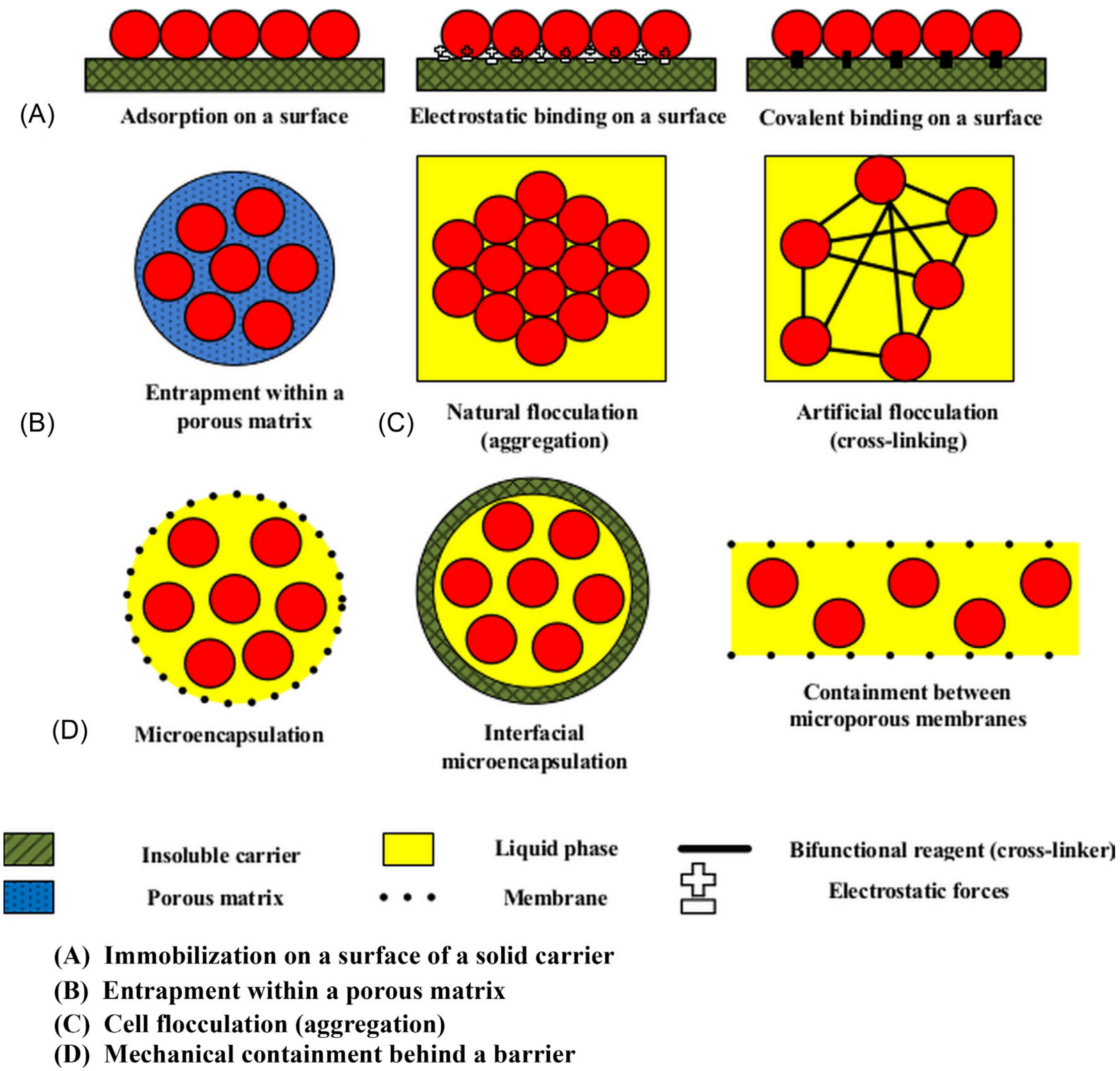

(A)
Adsorption on a surface
Electrostatic binding on a surface
Covalent binding on a surface
(B)
Entrapment within a porous matrix
(C)
Natural flocculation (aggregation)
Artificial flocculation (cross-linking)
(D)
Microencapsulation
Interfacial microencapsulation
Containment between microporous membranes
Insoluble carrier
Porous matrix
Liquid phase
Membrane
Bifunctional reagent (cross-linker)
Electrostatic forces
(A) Immobilization on a surface of a solid carrier
(B) Entrapment within a porous matrix
(C) Cell flocculation (aggregation)
(D) Mechanical containment behind a barrier

Fig. 13.7 Basic methods of immobilization of microbial cells (Kourkoutas et al., 2004; via www.sciencedirect.com).

from chemical heterogenic catalysis, can be used for this purpose. Another option is to adapt the free cell models using different types of coefficients, summarized in the practice as efficiency coefficients (Angelov et al., 2012; Willaert et al., 1996).

Substrates can reach the immobilized biocatalyst only by diffusion from the solution mass through the stationary liquid film on the surface of the carrier, and if the carrier is porous, by diffusion into its pores (Fig. 13.8).

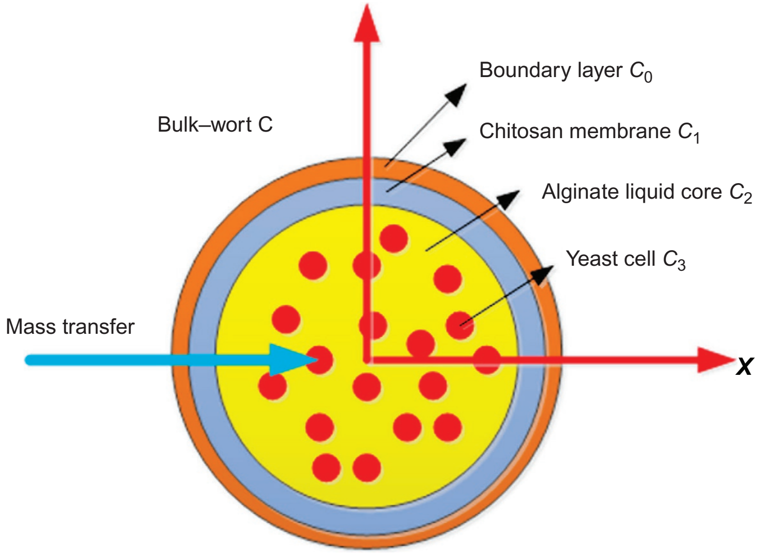


Fig. 13.8 Major diffusion resistances in immobilized cell systems (e.g., alginate/chitosan capsules with a solid or liquid core).

Depending on the ratio between the rates of the diffusion stages and the biocatalytic reactions, one of the following situations is observed:

- The catalytic process is controlled by external diffusion when reactions in the surface layer of the carrier particles occur faster than the rate of transfer of the substrates from the solution.
- If the overall process rate is limited by the diffusion of reagents in the pores of the carrier, the catalytic process is controlled by internal diffusion.

Immobilization is the cause of the creation of a microenvironment in which the mass exchange of the substrates is difficult compared to the case of freely suspended enzymes and cells. This is due to the introduced solid phase (carrier), which results in additional resistances at the liquid-solid boundary and in the interior of the particles.

The diffusion resistances have a great influence on the observed rate (V_E) of the catalyzed process by immobilized systems. The degree of V_E progress toward the maximum possible rate (V) of a reaction without transport limit is recorded by the efficiency factor (Angelov et al., 2012; Willaert et al., 1996):

$$\eta = \frac{V_E}{V} \tag{13.7}$$

13.5.1.1 External Diffusion Resistances

External mass transfer involves the transport of substrates to the surface of the carrier and the secretion of reaction products from it.

Regardless of the hydrodynamic conditions in the bioreactor, an unstirred liquid film is formed at the liquid-solid boundary. The transfer of the reagents is conducted extremely by molecular diffusion inside that film. Since molecular diffusion in the liquid takes place very slowly ($D = 10^{-6} - 10^{-5}\,\text{cm}^2/\text{s}$), the resistance of the liquid film can be a reason for limiting the process by the external mass transfer (Angelov et al., 2012; Willaert et al., 1996):

$$r_f = k_s a_s (S - S_s) \tag{13.8}$$

The mass-exchange coefficient k_s in the first approximation can be estimated from the dependence (Angelov et al., 2012; Willaert et al., 1996):

$$k_s = \frac{2D}{d_p} \tag{13.9}$$

The calculated value of k_s is generally lower than the actual value due to the effects of agitation and particle agglomeration. The determination of k_s can be performed using criteria dependencies of the type $Sh = f(Re, Sc)$ (Angelov et al., 2012; Willaert et al., 1996).

The influence of external mass exchange when the catalyzed reaction occurs in one of the phases is usually noted by comparing its overall rate and the rate of the reaction without a concentration gradient between the solution and the surface of the carrier. The external efficiency factor η_e is defined as the ratio of these two rates and is a function of the Damkohler criteria (Angelov et al., 2012; Willaert et al., 1996):

$$D_a = \frac{V_m}{r_{\max}} \tag{13.10}$$

When the mass exchange rate is significantly lower than the reaction rate ($D_a \gg 1$), the latter depends much less on the temperature and changes linearly with the area of the reaction surface (Angelov et al., 2012).

At steady state, the rate of mass exchange of the substrate from the solution is equal to the reaction rate at the nonporous biocatalyst surface:

$$k_s a_s (S - S_S) = \frac{V_m S_S}{(K_m + S_S)} \tag{13.11}$$

provided that the reaction kinetics is subjected to the Michaelis-Menten equation, respectively to the Monod equation (Angelov et al., 2012).

External diffusion resistances can be neglected in most fermentation systems due to the turbulence of streams caused by the agitation applied. This is not applicable to solid-layer immobilized biocatalyst systems (Angelov et al., 2012).

13.5.1.2 Internal Diffusion Resistances

In a number of cases, the rate of the reaction catalyzed by enzymes or cells immobilized inside a carrier may be dependent on the diffusion of the substrates in the matrix pores. The influence of internal diffusional resistances is usually noted by the efficiency factor η_i, which is a ratio of the observed response rate and the reaction rate calculated for a matrix ideally permeable to reagents. For immobilized cells, η_i is the relation of effective and true reaction kinetics (Angelov et al., 2012; Willaert et al., 1996).

The efficiency factor can be obtained by the co-solving of Fick's second law of diffusion of substrate into the pores and the equation of the kinetics of a reaction in a porous medium and applying Monod-based kinetics of the reaction (Angelov et al., 2012; Willaert et al., 1996):

$$\begin{aligned}
&D_e r^{-2}\frac{d\left(\dfrac{ds}{dr}\right)}{dr}=\frac{V_m S_S}{(K_m+S_S)}\\
&\frac{dS}{dr}=0\ \text{3a}\ r=0\\
&S=S_r\ \text{3a}\ r=R
\end{aligned} \tag{13.12}$$

The solution to Eq. (13.12) can be achieved numerically, producing graphs for the substrate profile in the matrix $S(r)$. Another way to solve the system is to bring it in a dimensionless form that uniquely characterizes the distribution of the substrate in the volume of the catalyst and the influence of the hydrodynamic environment in the bioreactor on the process micro-kinetics. This is the Thiele (Φ) modulus (known from the catalytic theory) (Eq. 13.13), which depends on the particle shape and the order of the catalyzed reaction. Data on some characteristic cases of solution with the help of the modulus Φ, which are solved graphically and are represented in the form of graphs for $\eta = f(\Phi)$ can be found in the specialized literature (Angelov et al., 2012; Willaert et al., 1996).

$$\frac{d}{dx_i}\left(x_i^2\frac{dC_i}{dx_i}\right)=\Phi_i^2 C_i \quad with\,\Phi_i=\sqrt{\frac{V_m X}{D_i K_{0.5}}} \tag{13.13}$$

For small values of Φ, the efficiency factor is practically equal to one for all substrate concentrations, that is, diffusion effects can be disregarded.

13.5.2 Models Used to Describe Alcohol Fermentation With Immobilized Cells

Data for modeling alcohol fermentation with immobilized cells in beer production are scarce in the literature. This is mainly because of the fact that the establishment of the impact of the two types of resistances in the system is complicated, and even impossible.

For this reason, there are two main options for assessing fermentation kinetics using immobilized cells:

- Application of free cell models and comparison of both types of fermentation using efficiency coefficients.
- Expanding the model applied to free cells with mass exchange equations and comparing the two groups of models using efficiency coefficients.

The first method is extremely effective, especially because comparisons for both types of fermentation can be made quickly and easily. Such an approach is used in the works of Parcunev et al. (2012), Vassilev et al. (2013), Naydenova et al. (2014), and Kostov (2015). In this series of publications, the authors studied different types of fermentation processes with free and immobilized cells in terms of primary and secondary metabolism, establishing different parameters in both processes. For this purpose, the kinetic equations (13.2)–(13.5) for free and immobilized cells are used, and the comparison is made using the efficiency coefficients that can be defined as follows:

$$\begin{aligned} \eta_{\mu} &= \frac{\mu_{\max}^{imm}}{\mu_{\max}^{free}} \\ \eta &= \eta_{ex}\eta_{in} \\ \eta_{q} &= \frac{q_{\max}^{imm}}{q_{\max}^{free}} \end{aligned} \tag{13.14}$$

This approach makes it possible to compare the rates of the two processes, and the accuracy of the models depends on the parameters already described. This in turn provides an advantage in optimizing the immobilized cell processes and detecting the overall influence of the diffusion resistances. The main disadvantage is that on the basis of the overall coefficient of efficiency it is difficult to differentiate the influence of the two types of resistances, external and internal. This problem can be eliminated if the influence of the two types of resistances is determined separately. This is usually done by providing suitable stirring conditions in the fermentation system to minimize the stationary film around the immobilized preparation, resulting in a minimal influence of the external diffusion resistances.

In some cases, even when choosing a method and carrier for immobilization and a fermentation system, it is clear that resistances will prevail. For example, in dense-bed reactors, due to the low agitation efficiency, external resistances will have a decisive influence on the process rate. The use of multilayer capsules leads to an increasing influence of internal resistances, mainly due to the fact that different layers have different diffusion coefficients.

Another approach for describing the diffusion resistances is used in the work of Petelkov et al. (2016). In this system, the differential equation system (13.15) is expanded by two equations, which include summarized dependencies of the mass exchange in the fermentation system (13.16). In contrast to the classical mass-exchange equations, in the dependencies (13.16), there is a biological part because the changes in the concentrations of the substrate and the ethanol are strongly influenced by the fermentation process rate. Hence, this results in the difficulty that the accuracy of the chosen model for describing the biological process will affect the model accuracy.

Most commonly, the two approaches cited are combined in a single model, which allows for a more accurate description of the fermentation process. The results of the identification of the parameters of such a fermentation process are presented in Table 13.1. These data can be discussed in the following way:

- The immobilized cell process proceeds at a rate 5.4–8.6 times as high as the rate of the free cell process. This in turn leads to shortening of the fermentation time by 24–48 h, depending on the initial wort extract.
- Immobilized cells are less affected by-product and substrate inhibition, as the K_{SX} and K_{SP} saturation constants are lower than those of the free cell processes.

$$\left|\begin{array}{l} \dfrac{dX}{d\tau} = \mu X \\ \dfrac{dP}{d\tau} = qX \\ \dfrac{dS}{d\tau} = -\dfrac{1}{Y_{X/S}}\dfrac{dX}{\tau} - \dfrac{1}{Y_{P/S}}\dfrac{dP}{d\tau} \\ \dfrac{dE}{d\tau} = Y_E \mu X \\ \dfrac{dFA}{d\tau} = Y_{FA} \mu X \\ \dfrac{dA}{d\tau} = Y_A \mu X - k_A XA \\ \dfrac{dVDK}{d\tau} = Y_{VDK} \mu X - k_{VDK} XVDK \end{array}\right. \tag{13.15}$$

$$\left|\begin{array}{l} \dfrac{dG_{im}}{dt} = K_{LS}\left(G - G_{im}\right) - \left(\dfrac{\mu X}{Y_{X/G}}\right) - \left(\dfrac{qX}{Y_{E/G}}\right) \\ \dfrac{dE_{im}}{dt} = -K_{LP}\left(E_{im} - E\right) + \left(\dfrac{qX}{Y_{E/G}}\right) \end{array}\right. \tag{13.16}$$

- The process of immobilization affects to varying degrees the synthesis and reduction of various metabolite groups:
  - Immobilized cells are characterized by increased ester formation ($\eta_E = 1.212$) and mildly reduced synthesis of higher alcohols ($\eta_{FA} = 0.805$), which is reflected in a higher concentration of esters and a lower concentration of higher alcohols in the finished beer obtained in the process with immobilized cells.
  - In the process with immobilized cells there is a lower carbonyl compound production rate ($\eta_A = 0.281$; $\eta_{VDK} = 0.646$), but a slower reduction ($\eta_{KA} = 0.368$; $\eta_{KVDK} = 0.439$). However, the reduction in the immobilized cell system occurs faster because of the increased volume of cells in the working volume compared to that in the free cell process.

Diffusion resistances are reflected by the two global coefficients of K_{LS} and K_{LP}. In the present case, they indicate that the process is more limited by substrate consumption, as K_{LS} has a twofold lower value than K_{LP}.

13.6 Control of Fermentation Processes

Fermentation is characterized by complex, biological, nonlinear phenomena, and specific process dynamics. That is why it is an important field of interest for system engineering. Since the primary goal of fermentation research is the cost-effective production of bio products, it is important to develop methods that allow production of the desired product in a high concentration, with high productivity and yield.

13.6.1 Methods for Control of Fermentation Processes

Depending on the applicability of a model for control design purpose, there are two main control strategies (Ignatova and Lyubenova, 2011):

(A) Model-based control: if the model is used for control design.

(B) Knowledge-based control: if no model is necessary, expert knowledge only and artificial intelligence (AI) methods are used for control design.

(C) Depending on the level of the control problems, the controlled variables (physicochemical only or biochemical as well), the control algorithms, etc., the control strategies could be divided into:

  (a) Classical automatic control: when classical control laws (such as P, PI, PD) are used.

  (b) Advanced process control: more sophisticated control algorithms are applied.

  (c) Hierarchical control: more than one level of control is available.

Fermentation processes are carried out using three modes of cultivation: batch, fed batch, and continuous mode. During the batch mode, only physicochemical parameters could be controlled. The feeding during fed-batch and continuous modes of cultivation allows biochemical variables to be controlled as well (Ignatova and Lyubenova, 2011).

Due to the nonstationary and nonlinear nature of fermentation processes and the lack of reproducibility of the experiments, a contemporary direction for increasing the fermentation process efficiency is the introduction of online adaptive control of the main biological variables: concentrations of biomass, substrate, and metabolic product. In the last two decades, a widely used approach to the design of advanced process control (nonlinear adaptive, robust, optimal, etc.) of fermentation processes is the so-called general dynamical model approach (GDM) (Bastin and Dochain, 1990). This approach is based on the GDM of the process. This model linearizes the closed control loop of nonlinear objects and allows the well-developed linear control theory (LCT) to be applied for solving the problems of model-based control design and the problem of online state and parameter estimation for a wide class of fermentation processes. The general principles of this approach are outlined in the following with some applications for specific alcohol fermentations.

13.6.2 Formalization of Kinetics for Monitoring Fermentation Process Control

13.6.2.1 General Dynamical Model Approach: Basic Information

The GDM of a bioprocess could be derived by the simplest description of the process: the process reaction scheme. In contrast to the common practice in chemical kinetics, the reaction scheme used for GDM derivation represents a qualitative relationship between the components dissolved in the culture broth. This allows the integration of chemical, biochemical, and microbial growth processes in a unified approach. The GDM is given in matrix form as follows (Bastin and Dochain, 1990):

$$\frac{\mathbf{d}\xi}{\mathbf{dt}} = \mathbf{K}\varphi(\xi) - D\xi + \boldsymbol{F} - \boldsymbol{Q} \tag{13.17}$$

The GDM consists of two main parts: process kinetics ($\mathbf{K}\varphi$) and transport dynamics ($D\xi - \boldsymbol{Q} + \boldsymbol{F}$). Usually the transport dynamics is well known and provides possibilities for process control design but the process kinetics is fully or partially unknown.

The model for φ is proposed. It is based on the following fact: the reaction can take place only if all the reactants are present in the reactor. In other words, the reaction rate is necessarily zero whenever the concentration of one of the reactants is zero.

- *Minimal modeling of reaction kinetics*

From an engineering viewpoint, an idea of minimal modeling of the reaction kinetics is formalized as follows:

$$\varphi(\xi)=\mathbf{H}(\xi)\rho(\xi) \tag{13.18}$$

where $\mathbf{H}(\xi)$—a Mxr matrix of known function of $\boldsymbol{\xi}$; $\boldsymbol{\rho}(\xi)$—a vector of unknown function of ξ with dim $\rho(\xi)=r$.

The idea is to insert into $\mathbf{H}(\xi)$ only the prior knowledge which is available regarding kinetics and ρ is considered a completely unknown time varying parameter.

When there is no prior knowledge about the kinetics, it is considered as fully unknown time varying parameter:

$$\rho(\xi)=\varphi(\xi)\text{and}\,\mathbf{H}(\xi)=\mathbf{I_M} \tag{13.19}$$

This case is investigated by Ignatova and Lyubenova (2011) and Lyubenova and Kostov (2016) and applied for software sensors (SS) and adaptive linearizing control design for alcohol fermentation processes.

- *SS design*

SS are algorithms that estimate immeasurable process variables and kinetic parameters based on measurable variables functionally connected with the immeasurable ones. Since the lack of reproductivity of experiments and promising sensors are main characteristics of these processes, the monitoring of fermentation processes in real time is the object of intensive research in the last decades (Bastin and Dochain, 1990; Ignatova and Lyubenova, 2011; Lyubenova et al., 2012; Lyubenova and Kostov, 2016). The development of SS makes it possible to follow all physiological states of the process. SS are used in adaptive control algorithm design related to the enhancement of the quality and quantity of the target product. The role of SS is demonstrated by the examples in the following.

- *Adaptive linearizing control*

In Bastin and Dochain (1990), the conception of exact linearizing control approach was proposed. The main difference between conventional and linearizing control relies on the way of introducing linearization in the problem. In a standard approach, one first calculates a linearized approximation of the nonlinear biochemical model, and then one designs a linear controller for this approximated model. But the closed loop remains nonlinear and guaranteed to be stabilized only locally but not on a wide range of operating points. In the exact linearizing control approach, one obtains a nonlinear controller which is precisely designed to achieve a linear closed loop which is unconditionally stable whatever the operating point or the transient fed-batch trajectory. This nonlinear controller is designed on the basis of GDM and the reference model well known from LCT. The principle of linearizing control is to find a control law which is a multivariable nonlinear function of ξ (ξ_m, ξ_{unm}), $\boldsymbol{Q}$, $\boldsymbol{F}$. Variables ξ_m are measured online but unmeasured variables ξ_{unm} and unknown kinetics information have to be received by SS.

13.6.2.2 Adaptive Control of Continuous Alcohol Fermentation

The proposed fermentation management methodology can be applied to various fermentation processes: batch, fed-batch, and continuous processes. The work of Lubenova et al. (2013) demonstrates the application of the proposed methodology for continuous alcohol fermentation. For this purpose, the nonstructural model (13.15) is used, and it is supplemented with the mass-exchange equations (13.16).

Identification of the model parameters is conducted using experimental data of each batch fermentation. An optimization procedure based on evolutionary algorithm is applied. The optimization criterion is chosen as to obtain the minimal mean square error between model and experimental data of a specific fermentation. Parameter identification is done using Matlab 7.0 Math works, USA. The optimal values of the model parameters for the three chosen immobilized cell fermentations are given in Kostov et al. (2013).

13.6.2.3 SS for Ethanol Production Rate

Measurements of ethanol concentration are used as input for the SS. The output is the ethanol production rate, R_E, considered as unknown time-varying parameter. This sensor can be written as (Kostov et al., 2015; Lyubenova and Kostov, 2016):

$$\frac{d\hat{E}}{dt} = \hat{R}_E - DE + C_1\left(E - \hat{E}\right) \tag{13.20a}$$

$$\frac{d\hat{R}_E}{dt} = C_2\left(E - \hat{E}\right) \tag{13.20b}$$

The first equation of the sensor presents a copy of the input-output equation and a correction term, which is a product of the estimation error and a tuning parameter C_1. The second equation is an update equation for the ethanol production rate including the estimation error multiplied by a tuning parameter C_2. For the system (13.20), the dynamics of the observation error $\left(e = E - \hat{E}\right)$ and tracking error $\left(\rho = R_E - \hat{R}_E\right)$ is presented by the following system:

$$\frac{d}{dt}\begin{bmatrix} e \\ \rho \end{bmatrix} = \begin{bmatrix} -C_1 & 1 \\ -C_2 & 0 \end{bmatrix}\begin{bmatrix} e \\ \rho \end{bmatrix} + \begin{bmatrix} 0 \\ \dfrac{dR_E}{dt} \end{bmatrix}$$

The tuning parameters C_1 and C_2 are calculated as functions of the eigenvalues, p_o, of this error system. In order for the estimator (13.19) to be stable, the eigenvalues must lie in the left half plane. The following equations present relationships between the tuning parameters and the two eigenvalues selected to be equal to each other p_o:

$$C_1 = -2p_o \quad C_2 = p_o^2 \tag{13.20c}$$

where p_o, C_1 and C_2 are constants.

13.6.2.4 Adaptive Control Design

Following the procedure for adaptive linearizing control design (Bastin and Dochain, 1990), the control algorithm for the considered process is derived as

$$D = \frac{-\lambda\left(E^* - E\right) + \hat{R}_E}{E} \tag{13.21}$$

where λ is the control tuning parameter; $\hat{R}_E$ the estimation values of ethanol production rate received by estimator (13.3); E the measured value of ethanol concentration in the culture medium; and E^* the set point of ethanol concentration corresponding to maximal ethanol production rate reached in batch phase.

The investigations of SS (13.20) during the batch phase of the three experiments mentioned above are presented in Fig. 13.9. The tuning parameters C_1 and C_2 are calculated using the relationship (13.20c) with $p_o = -2$. This value is chosen as a compromise between the rate of convergence and the disturbance sensibility of the estimation algorithm (13.20).

For simulation investigations of the adaptive control (13.21), the scheme in Fig. 13.10 is used. The value of the control tuning parameter λ is set to be 0.1. The control simulation results for the three experiments are compared in Figs. 13.11–13.13. An advantage of the preliminary investigations of the process by models is the possibility to receive information about the values of the substrate and the ethanol concentrations in the beads as shown in Fig. 13.12.

Figs. 13.11C and 13.12B show that ethanol concentrations guarantee a constant productivity of the process keeping the necessary difference ($E_{im} - E$) for permanent diffusion of the ethanol produced by the cells and secreted into the culture medium. The chosen concentration of substrate in the feed, S_{in}, leads to steady state with almost full substrate depletion after period 3/D which is the optimal case from an expert's point of view. Observing the time elapse of substrate concentration in the beads, it decreases slowly and becomes almost zero for all processes in steady state (Fig. 13.12A). In Fig. 13.13, the ethanol production rate and control input—dilution rate are plotted.

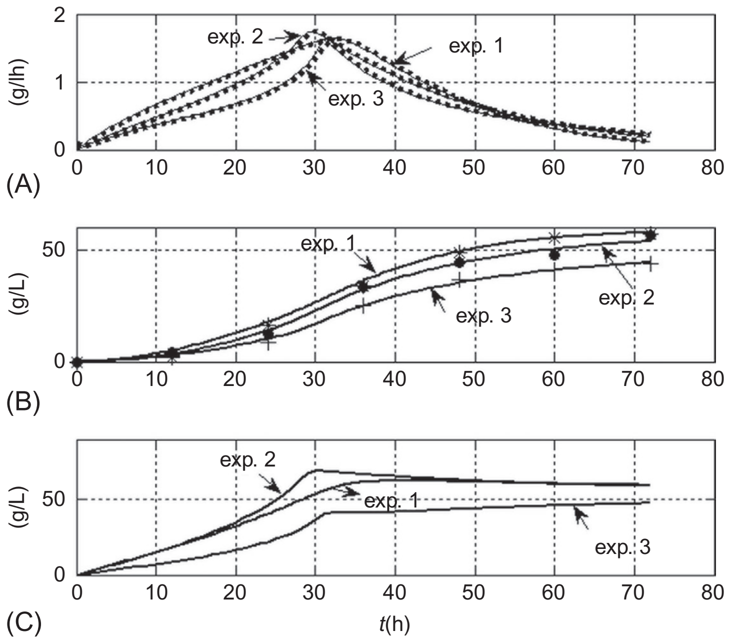


Fig. 13.9 Batch fermentation—model *(solid lines)*; exp. 1 (*), exp. 2 (•), exp. 3(+); ethanol production rates' estimates (…). (A) Ethanol production rate; (B) ethanol in the culture medium; (C) ethanol in the beads.

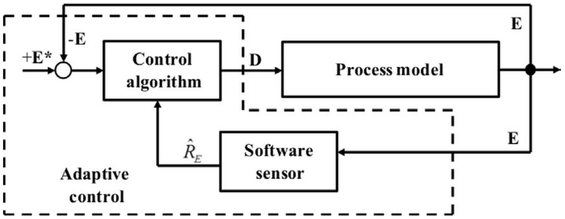


Fig. 13.10 Control scheme.

For the investigated processes, the ethanol production rate parameter, R_E, has a maximum, which appears between the 30th and the 36th hour depending on the fermentation (Fig. 13.12a). At the same time, the control set-point, E^*, has the value of ethanol concentration corresponding to the maximum of R_E and a switching from batch to continuous mode of cultivation is realized automatically. Then, this parameter decreases slowly and reaches a steady-state value.

The proposed example demonstrates the possibility to design continuous control starting from experiments in a batch mode of cultivation. The simulation investigations of the control strategy stabilize the ethanol concentration in the culture medium to a value that would guarantee a maximum ethanol production rate. The derived

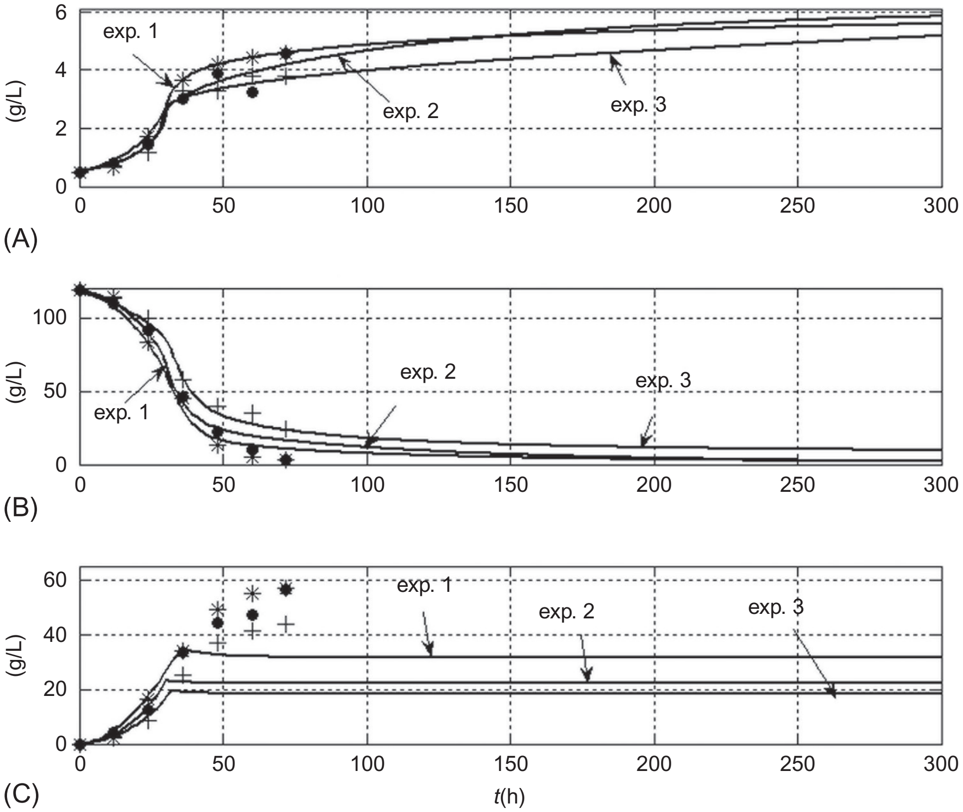


Fig. 13.11 Continuous fermentation. (A) Biomass; (B) substrate in the culture medium; (C) ethanol in the culture medium.

algorithms are simple enough and could be applied in laboratory conditions using LABVIEW system.

13.7 Conclusion

This chapter provides a critical analysis of existing kinetic models for describing the alcohol fermentation process in brewing. The analysis was based on the knowledge of the microbiology and biochemistry of the fermentation process as well as on real data from fermentations. The purpose of this analysis is to allow other researchers to choose the most appropriate model for their work. An analysis of the diffusion resistances existing in the immobilized cell systems and their influence on the fermentation process kinetics was also described. At the end of the work, basic approaches to the control of the fermentation process have been presented, as well as the innovative approach of a dynamic model for the fermentation process control.

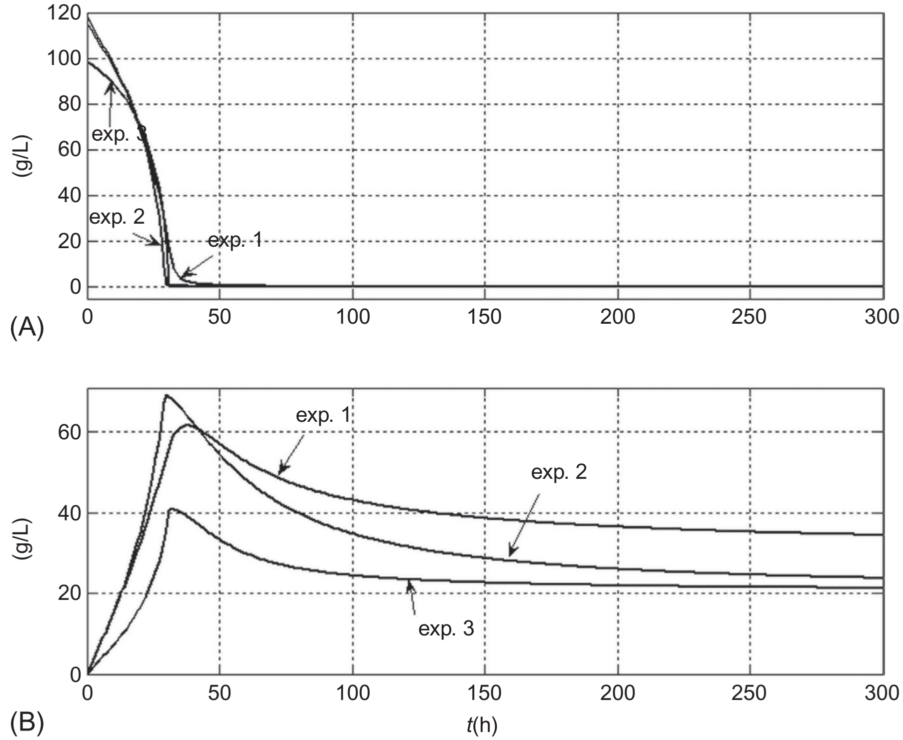


Fig. 13.12 Continuous fermentation—concentrations in the beads. (A) Glucose in the beads; (B) ethanol in the beads.

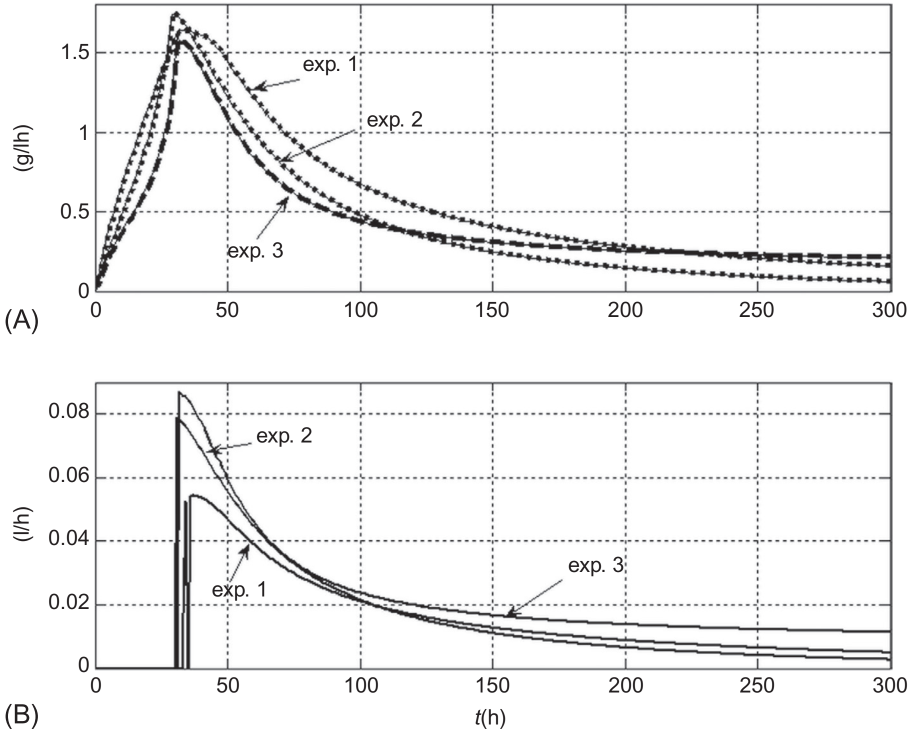


Fig. 13.13 Continuous fermentation: (A) ethanol production rate and (B) dilution rate-control input.

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14

NEW ALCOHOLIC FERMENTED BEVERAGES—POTENTIALS AND CHALLENGES

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14.1 Introduction

The production of alcoholic beverages has been carried out by humanity for more than 5000 years. With emphasis on grape wine and beer, in terms of produced and commercialized volume, the market of alcoholic beverages has grown annually and constantly demanded more investments in innovative technologies. In addition to the worldwide produced and marketed beverages, in recent decades, there has been an increasing interest in nontraditional beverages that are not widely marketed. These beverages are gaining prominence and consequently more attention has been devoted to improving production processes and the quality of these beverages. Also, in recent years, there has been a growing search for the development of new beverages elaborated from the most varied substrates.

The growing interest in new and nontraditional beverages has been motivated by several reasons, among which we can cite the need to use surplus and production; the use of products without quality standard, as in the case of some fruits; better exploration of sub-exploited potential as in the case of native fruits of temperate and tropical climate; adding value to production chains as in the case of by-products of coffee processing and the dairy industry; and the necessity to create "new" destination for substrates generated from plants that are not yet fully exploited, as in the case of sweet sorghum. In this context, many studies (Table 14.1) have been developed in recent years aimed to produce new alcoholic fermented and distilled beverages from differentiated and sub-exploited substrates.

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Table 14.1 Beverages and Substrates Used in Their Production

Beverage	Substrate	References
Spirit	Spent coffee ground	Sampaio et al. (2013)
Spirit	Fluid cheese whey	Dragone et al. (2009)
Spirit	Powder cheese whey	Dragone et al. (2009)
Wine	Mango	Reddy and Reddy (2005)
Spirit	Jabuticaba	Duarte et al. (2011), Gonçalves and de Souza (2014)
Wine	Papaya	Umeh and Udemezue (2015), Lee et al. (2013)
Wine	Raspberry	Duarte et al. (2010)
Spirit	Raspberry	González et al. (2011)
Wine	Orange	Okunowo et al. (2005), Selli et al. (2003)
Spirit	Orange	Santos et al. (2013)
Wine	Pineapple	Dellacassa et al. (2017), Okeke et al. (2015)
Beer	Sweet sorghum	Oriola et al. (2017), Claver et al. (2011)
Spirit	Sweet sorghum	Zheng et al. (2012), Xing-Lin et al. (2017), Guimarães (2013)

In this chapter, we report several examples on the use of fruits, by-products of coffee processing, cheese whey, and sweet sorghum in the production of fermented alcoholic beverages, highlighting some aspects related to the substrates, to the beverages, besides the existing challenges and potentials.

14.2 Alcoholic Fermented Beverages From Coffee By-Products

In recent years, the beverage industry has shown great interest in the development of new products from unconventional raw materials that can confer differentiated flavors to the produced beverages, aiming to attract new consumers. Among these new substrates, by-products from coffee processing are promising alternatives due to their chemical and sensorial qualities. The industrial processing of the coffee beans is carried out to obtain the coffee in the form of powder, by means of the removal of the husk and the mucilaginous portion of the grain. Among the products generated are pulp, husk, silver skin and residual water, a by-product rich in suspended solids and dissolved solids (Selvamurugan et al., 2010), and the solid residue from coffee extraction (spent coffee grounds).

Considering that coffee consumption is a daily practice in many countries, the trade and use of this grain has continuously increased worldwide, generating huge amounts of solid waste from its production, roasting, and consumption. Most processed coffee is discarded in the form of sludge, resulting from paper filtering or the percolation process in coffee machines. This material does not have a commercial value, being preferentially discarded as solid waste or sent to composting (Zuorro and Lavecchia, 2012), constituting a source of contamination, and representing a serious environmental problem.

In general, large-scale management of coffee by-products continues to be a challenge worldwide due to its caffeine content, free phenols and tannins (polyphenols), substances mainly present in pulp, but also in husk and wastewater, which are known for their toxicity to most living things (Enden et al., 2002; Selvamurugan et al., 2010; Murthy and Naidu, 2012).

According to Murthy and Naidu (2012), the rich nutritional composition of these by-products allows their conversion, through the action of microorganisms, into different products of commercial value such as acids (citric and gibberellic), bioactive compounds, volatile aromatic compounds, pigments (carotenoids), microbial biomass (edible mushrooms), enzymes (amylase, pectinase, protease, and xylanase), and ethanol, which can also be produced in the form of alcoholic beverages.

The aromatic properties of coffee processing by-products also constitute a quality that makes them suitable candidates for application in alcoholic fermentation to produce beverages. Due to the presence of aromatic compounds, which confer positive flavor and aroma characteristics, coffee by-products such as spent coffee grounds, pulp, and coffee husk may also be used for the production of coffee-flavored spirits. Although promising, the development of these beverages still presents a series of challenges, such as the search for yeast strains capable of the best use of the substrate, the selection of the best methods for the treatment of the by-product in order to maximize the consumption of available carbohydrates and the extraction of the volatile compounds, and the adjustment of the fermentation parameters aiming at the best final quality of the beverages.

14.2.1 Coffee By-Products With Potential to Be Used in the Production of Distilled Beverages

The industrial processing of the coffee beans, made to obtain the coffee powder, consists in the removal of the husk and the mucilaginous portion of the grain. Murthy and Naidu (2012) state that the by-products commonly generated during the processing step vary according to the type of process applied, which may be dry or wet, and the roasting and fermentation steps. Among the by-products produced, pulp, husk, and coffee grounds constitute some of the substrates suitable for the production of alcoholic beverages.

The coffee pulp is the by-product generated from the processing of the coffee by wet process, while a coffee husk is the by-product generated from the dry processing. These both by-products are rich in organic matter and nutrients, but contain caffeine, tannins, and phenolic compounds (Franca and Leandro, 2016), which can act by inhibiting the microbial metabolism and, consequently, affecting a fermentation.

The spent coffee grounds are characterized by the high organic content, present in the form of insoluble polysaccharides, fatty acids, amino acids, polyphenols, and minerals (Campos-Vega et al., 2015; Kourmentza et al., 2018). Among other properties are its high humidity (around 80%–85%), high sugar content, mostly mannose and galactose, and the significant fraction of proteins (Mussatto et al., 2011).

Finally, the coffee husk is the raw material obtained when coffee beans are processed via dry method. With a high carbohydrate content, it also has caffeine, tannins, and other polyphenols in its constitution (Pandey et al., 2000). The higher carbohydrate content makes it a potential substrate for the fermentation process for the generation of ethanol.

Considering the composition of above-described coffee by-products, especially their sugars content, among which are pentoses and hexoses, different microorganisms can be used in the conversion of these carbohydrates into ethanol even in the form of alcoholic beverages. According to Mussatto et al. (2012), *Saccharomyces cerevisiae* is the most widely used microorganism for this purpose, due to its ability to grow in medium containing high sugar concentration producing high ethanol amounts, although it is only capable of fermenting hexoses. The yeasts of the genus *Pichia, Candida, Schizosaccharomyces*, and *Pachysolen*, filamentous fungi *Paecilomyces, Mucor, Monilia, Fusarium*, and *Clostridium*, and bacteria such as *Bacillus*, *Thermoanaerobacter*, and *Erwinia*, are reported for the production of ethanol from pentoses obtained by hydrolysis of hemicellulosic material, characteristic of some coffee by-products (Hahn-Hägerdal et al., 1994; Millati et al., 2005). For fermentation of lignocellulosic hydrolysates, *S. cerevisiae* and *Kluyveromyces fragilis* were able to ferment different sugars for the production of ethanol (Dragone et al., 2011; Tehrani et al., 2015). Thus, it is widely known that the presence of sugars in the by-products generated in the coffee processing allows the conversion of these substrates into ethanol in the form of fuel, destined for use in the transport sector. However, recently the generation of ethanol for the distillate industry, such as the production of spirits, has been a new application of the fermentation process with the use of these by-products.

14.2.2 Production of Spirits From Coffee By-Products

The spirit is a distilled alcoholic beverage produced from the fermentation of agricultural products containing carbohydrates (Christoph and Bauer-Christoph, 2007). The production of alcoholic beverages such as spirits using agricultural by-products from agro-industrial activities presents great potential due to the low cost of the raw material, to offer characteristic aromas to the beverages and to provide sugars necessary for the fermentation (Sampaio et al., 2013).

In this context, among the agricultural by-products, those from coffee processing also represent potential raw materials for the production of distilled beverages. In particular, the pulp and the coffee husk, obtained respectively by the wet and dry processing of the coffee, can be suitable substrates for microbiological processes aiming at the generation beverages with unique aromatic characteristics. The compounds responsible for the beverage's aroma, its levels, odor attributes, and thresholds are important characteristics for the quality and authenticity of spirits (Christoph and Bauer-Christoph, 2007).

The potential of coffee pulp for alcoholic fermentation was recently demonstrated by our group in the work of Bonilla-Hermosa et al. (2014). For the alcoholic fermentation, a medium containing 10% coffee pulp in coffee wastewater was prepared and fermented with different yeast as *S. cerevisiae, Pichia anomala, P. guilliermondii, Torulaspora delbrueckii, K. marxianus*, and *Hanseniaspora uvarum*. After this fermentation, the yeast *H. uvarum* showed highest potential to convert sugars from coffee by-products into ethanol. Using this yeast, we also proposed a fermentation with optimized substrate containing 12% of coffee pulp in wastewater supplemented with 1 g/L of yeast extract. These fermentations were carried out at 27°C and 105 rpm. After the first fermentation that last 24–48 h, depending on the used yeast, the amounts of ethanol ranged from 10.79 g/L for *P. anomala* to 14.67 g/L *H. uvarum*. The only one *S. ceversiae* used in this work, called CA11 and commercialized in Brazil form sugar cane spirit production, resulted in an ethanol content of 13.47 g/L. The fermented must obtained with different yeasts cited above, once analyzed by gas chromatographic, were characterized by the presence of different volatile aromatic compounds including 11 higher alcohols, six acetates, six terpenes, five volatile fatty acids, four aldehydes, and one ethyl ester.

It is worth noting that under optimized conditions, *H. uvarum* produced a fermented must with volatile compounds such as 2-phenylethanol, an aromatic alcohol with a rose-like odor; phenylethyl acetate with rose, honey, and apple odors; linalool with sweet, fresh, citrus odors; and b-citronellol with fresh, rose-like odors descriptors. Volatile compounds such as those mentioned above are very impactful in the aromatic characteristics of fermented alcoholic beverages demonstrating the feasibility of the use of coffee pulp to produce this kind of beverages.

Recently, we worked on the optimization of a methodology to produce a spirit using coffee pulp supplemented with different commercial sugars source aiming to increase the final ethanol content of the beverage. The must composed of 10% of coffee pulp added to commercial sucrose fermented with *S. cerevisiae* CA11 was distillated resulting in a spirit with high sensorial acceptance and coffee aromatic notes.

Another example of the coffee by-products potential was reported by Sampaio et al. (2013). In this work, the authors used spent coffee grounds as source of aromatic compounds and after a hydrothermal process where spent coffee ground was heat treated for 45 min at 163°C, the extract was supplemented with 180 g/L of sucrose. The coffee spent grounds extract was fermented by *S. cerevisiae* at 30°C/150 rpm. The fermented must was distilled resulting in a spirit characterized by the aromatic volatile compounds such as 3-methyl-1-butanol and 2-methyl-1-propanol, ethyl butanoate, ethyl hexanoate, ethyl octanoate, and phenylethyl acetate. The aromatic profile led the trained tasters to characterize the beverage with aroma descriptors such as coffee, frankly, and elegance reinforcing the feasibility of using spent coffee ground extract to produce a distilled beverage. In addition to the possibility of generating a value-added product as described earlier, the use of spent coffee ground in alcoholic fermentation is also feasible because of the large amount produced worldwide and also due its toxic character to the environment (Tehrani et al., 2015).

14.2.3 Challenges in the Use of Coffee By-Products for Beverages Production

Despite all the promising works and results described earlier, concerning to the use of coffee by-products for the production of new alcoholic beverages, some aspects related to the full development of technology and commercial product generation still need to be evaluated and improved.

Characteristics related to the chemical composition of the coffee bean and, consequently, of its by-products, represent a primordial aspect for the final quality of the beverage. As reported by Murthy and Naidu (2012), it is important to consider the differences between plants in the same cultivar and also the variation in different parts of the same individual. In addition, this composition also varies according to the geographic location of the plantation, the age of the plant, and the limitations and conditions of the soil in which the coffee is being cultivated.

In addition to the intrinsic aspects of coffee, the selection of more efficient fermentation strategies for the production of the coffee by-products-based beverage is also essential for the process. Among the main challenges, we highlight the choice of the most efficient microorganisms for fermentation, the method for pretreatment of the substrate, including the selection of the method of extraction of aromatic compounds that provides exploration use of the sensorial aspects of coffee to be passed to fermented beverages produced.

14.3 Alcoholic Fermented Beverages From Cheese Whey

14.3.1 Cheese Whey

Cheese whey is a liquid obtained after the precipitation of milk casein in the cheese making process, it is of great importance in the dairy industry due to the large volume generated and its nutritional composition. About 90% of the volume used for cheese production is converted to whey, which retains approximately 55% of the nutrients contained in milk (Dragone et al., 2009). Among the nutrients found in the cheese whey, the largest portion corresponds to lactose 39–60 kg/m^3, protein and mineral salts, 6–8 kg/m^3, lipids 4–5 kg/m^3, and dry extract 8%–10% (Dragone et al., 2011). These parameters make cheese whey a valuable product with applications in the food and pharmaceutical industries. However, the characteristics of cheese whey will depend on the quality of milk, animal, breed, animal feed, health, lactation, and among other factors (Prazeres et al., 2012).

Annually in the world is produced about 24,000,000 tons of cheese, which will result in approximately 21,600,000 tons of cheese whey. Due to the high biological oxygen demand (BOD) of cheese whey caused by the high concentration of lactose, this by-product becomes a major environmental problem of dairy industry, and can be 100 times more polluting than domestic sewage. In order to comply with environmental legislation, regarding the incorrect disposal of cheese whey, the industries have been looking for alternatives for its reuse. Most industries have been using cheese whey, and turning it into whey powder, whey protein concentrates, lactose, and mixed beverage such as fermented milk added of cheese whey. Another option is the use of fermentative processes to obtain value-added products, such as unicellular proteins, alcohols (ethanol and butanol), vitamins, organic acids (lactic acid, succinic, and propionic acid), among others (Mollea et al., 2013; Dragone et al., 2009). In these products, the concentrated cheese whey is more frequently used due to its high nutrients and lactose content, which, in the case of fermentation, helps the microbial growth.

14.3.2 Cheese Whey-Based Alcoholic Beverages

The cheese whey can be found in liquid or powder forms on the market. Cheese whey, which is obtained directly from cheese making is a concentrated whey goes through a process of ultrafiltration, nanofiltration, or other process that concentrates the nutrients present through the removal of excess water. Different works have been performed using substrates prepared from cheese whey powder or from, mainly, concentrated liquid cheese whey. Which of the cheese whey to use for an alcoholic fermentation will depend on the product to be obtained and on other factors such as the availability of the cheese whey itself. Fluid unconcentrated cheese whey has a low concentration of lactose when compared to concentrated cheese whey and whey powder, which can originate beverages with low alcohol content. The concentrated cheese whey and whey powder have a lactose content of approximately 60–70 g/L, which favors the production of alcohol during the fermentation process, making these two forms more used to obtain fermented alcoholic beverages, especially distilled ones. Considering the characteristics of whey as a concentration of sugars and nutrients and their world production, it can undoubtedly be considered as interesting substrate for alcoholic fermentation.

The fermentation of the cheese whey can be performed continuously or discontinuously. In both cases, the pH of the cheese whey can be adjusted with addition of acids or bases, and may range from 4 to 5.5 depending on the type of the selected whey and the selected yeast. The addition of other ingredients such as sweeteners, flavorings, and even fruit pulps can be carried out with the aim of improving the sensorial characteristics of the final product. Recently, we have tried to produce cheese whey-based beverages adding different vegetable juices and, the carrot juice demonstrated a good potential to improve sensory aspects of the beverage produced. The addition of fruit pulp helps to mask the smell of "cooked milk" present in the cheese whey (Chavan et al., 2015). The temperature used for cheese whey fermentation is frequently between 28°C and 30°C because almost all the fermentations are performed with *Kluyveromyces* yeasts.

Dragone et al. (2009) reported the production of a cheese distilled beverage where they used a continuous fermentation of pasteurized fluid whey with *K. marxianus*. The distillate presented an alcoholic content of 35.4% v/v and, 40 volatile compounds including alcohols, esters, acids, and aldehydes. Among the higher alcohols, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1-propanol, and 1-propanol were the most abundant. In the esters group, although ethyl acetate was the most abundant with 138 g/L. However, others aroma impacting esters such as ethyl butanoate, ethyl hexanoate, and ethyl octanoate, whose aroma descriptors include fruity, banana, and apple were also detected. The composition of the produced beverage allowed the authors to conclude the feasibility of using cheese whey in a continuous fermentation to produce a whey-based spirit. Dragone et al. (2009) worked with cheese whey powder to produce a distillated beverage using the yeast *K. fragilis* and a batch fermentation. The must for fermentation was prepared with lactose final concentration of 200 g/L fermented at 35°C/150 rpm for 92 h. The resulting beverage was characterized in sensorial analysis by aroma descriptors such as toasted, smoked, tried fruit, whiskey-milk cream, and dairy. Based on the obtained results, the authors showed that the cheese whey must with high initial lactose content can be used to produce a spirit with pleasant aroma and taste.

14.3.3 Challenges in the Use of Cheese Whey for Beverages Production

One of the greatest challenges in the use of cheese whey is its perishability, a fact that requires its rapid use or storage at low temperatures (<10°C or freezing) for the shortest possible period. Another challenge encountered for the use of cheese whey in the production of alcoholic fermented beverages concerns to lactose fermenting yeasts. Studies indicate that only 2% of the known yeast species are capable of fermenting lactose, among them are the yeasts of the genus *Kluyveromyces*, which has been widely evaluated for the production of ethanol, alcoholic beverages, enzymes, and aromatic compounds from fermentation of cheese whey. To use of yeasts that do not have the capacity to ferment lactose, this carbohydrate should be hydrolyzed by the enzymatic action of β-galactosidase known commonly as lactase. Recently, we worked with the alcoholic fermentation of the cheese whey using two yeasts. First, an inoculum of *K. lactis* for lactose hydrolysis during 48 h and after that, *T. delbrueckii* was inoculated to complete the fermentation by consuming of the monosaccharides, mainly that from lactose hydrolysis.

The choice of the most appropriated microorganism for the fermentation is a crucial step for the valorization of the cheese whey, since this microorganism will determine the fermentation time, the ethanol concentration, and the aromatic compounds generated, which will directly influence the quality of the produced beverage.

14.4 Alcoholic Fermented Fruits Beverages

In recent years, there has been a growing number of works reporting the production of alcoholic beverages based on fruits other than grape. Among these fruits, we can mention orange, mango, raspberry, pineapple, and papaya that are used for both wine and distillate production. The justifications for the use of these fruits include facts such as reuse of overproduction, use of fruit without quality standard for *in natura* consumption, and the generation of beverages with aromas and flavors characteristic of the used fruit.

Fruit wines are defined as undiluted alcoholic beverages, made with fruits others than grape, which are nutritious, tastier, and lighter stimulants since they contain most of the nutrients present in original fruits. These fruit wines undergo a period of fermentation and aging, and generally have an alcohol content between 5% and 13%, 2%–3% of sugars with an energetic value between 70 and 90 kcal per 100 mL. No other beverages, except water and milk, have gained such extensive acceptance throughout the years as wine. It is a beverage with a fresh fruit flavor that can be stored and transported under ambient conditions. Another relevant aspect is the nutritional value of the wine that is increased as a result of the release of amino acids and other nutrients during fermentation (Swami et al., 2014). Besides the production of fruit wines, as mentioned, many different fruits have been also used for the production of spirits. Different form wines, the process required to produce the fruit distillate is sometimes somewhat more complex once it involves several factors that influence the quality of the final product, such as the fruit pectin content and its correlation with methanol in the distillate. Below, we present a brief approach on the use of fruit in the production of fermented alcoholic beverages.

14.4.1 Production of Fruit Wines

The production of fruit wines has shown to be viable for several reasons, including the possibility to extract good quantities of juices from various tropical and subtropical fruits, including apples, pears, apricots, raspberries, peaches, cherries, oranges, mangoes, bananas, and pineapples, which can be fermented and processed into wines with peculiar flavors and aromas. The techniques used for the production of other fruit wines closely resemble the production of wines made from white and red grapes. The differences arise from two facts. It is slightly more difficult to extract sugar and other soluble materials from the pulp of some fruits, and the juices obtained from most fruits have a lower sugar content and higher acidity than the grapes. The use of specialized equipment to completely fragment or disintegrate the fruits, such as berries, followed by the extraction of the juice from the pulp, solves the first problem. Dilution of excess acid with water and addition of sugar to correct sugar deficiency solves the second problem.

The alcoholic fruit fermentation has been performed both spontaneously and with the use of selected starter cultures. As wine grape wine, spontaneous fermentation of the fruits is driven by complex and dynamic changes between communities of microorganisms involving bacteria and mainly yeasts. However, selected yeasts have been used in order to obtain faster, more reliable fermentations, reducing the risk of bacterial contamination and slow fermentation. As a main advantage, the use of selected yeasts allows rapid fermentation, low risk of contamination, better uniformity in fermentation rates, low competition for nutrients, higher beverage yield, low residual sugar concentrations, desirable flavor compounds, and maintenance of the sensory qualities of the beverage.

The *S. cerevisiae* strains are constantly evaluated aiming to improve fermentation, as well as improving the sensory qualities of beverages (Santos et al., 2013). However, the study of the ecology of natural yeasts from a spontaneous fermentation of fruit juices can provide fundamental knowledge yeast microbiota that is associated with fermentation of specifically fruit allowing the selection and development specific starter cultures and their functions in fermentations and roles in fruit wine flavors.

As well as in the production of grape wine, *S. cerevisiae* predominates in the fermentation of most fruits because of its ability to tolerate fermentation conditions such as high initial sugar concentration, high acidity, variations in temperature, availability of nutrients and, especially, increasing ethanol content. Nevertheless, other microorganisms such as bacteria and non-*Saccharomyces* yeasts are also used for the fermentation process and may contribute to the formation of the flavor of fruit wine. In recent years, the use of non-*Saccharomyces* yeasts has been recognized for its significant contributions and desirable effects on the sensory characteristics of fruit wine through the quantitative and qualitative diversity of fermentation products and by-products. However, these non-*Saccharomyces* yeasts are not vigorous or competitive microorganisms under fermentative conditions being frequently employed as adjuvant cultures in with strains of *S. cerevisiae*. Indeed, the use of mixed initiators in winemaking increased the complexity of wine flavors and had advantages over spontaneous and pure fermentations of *S. cerevisiae* (Rodríguez et al., 2010). There are several studies that report the use of mixed cultures of *S. cerevisiae* and other microorganisms in the production of fermented alcoholic beverages (such as fruit wines) or distilled (tequilas, cachaças, and fruit spirits). However, impacts on wine aroma and quality are directly influenced by substrate, rates and inoculation strategies, yeast species, and interspecific interactions (Lee et al., 2013).

Post-fermentation practices are performed after the fermentation has reached the desired stage or when the fermentation is complete. The wine is withdrawn from the yeast, usually in stainless steel vats or in oak barrels. During the storage period, the wine can go through several processes such as filtration, cold stabilization, blended, and/or clarification are some of these processes. Clarification may be achieved by storage, filtration, and/or centrifugation. Various clearing agents may be added to assist in the clarification of wines, such as enzymes, bentonite, diatomaceous earth, and egg albumin. The wine undergoes continuous changes during maturation and, at an appropriate stage, it is filtered and bottled.

14.4.1.1 Mango Wine

Mango (*Mangifera indica* L., Family-Anacardiaceae) is one of the most popular tropical fruits grown and consumed in the world. World production of mango is estimated at more than 117 tons per year, ranking fifth in total production among the world's major fruit crops. India ranks first among the world's mango producing countries, accounting for 54.2% of total mango produced worldwide. One of the ways to take advantage of the surplus mango production is the production of wine from this fruit, since mango juice is suitable for wine production because it contains bioactive compounds such as proteins, vitamins, and minerals (Chakraborty et al., 2014).

To produce mango wine, the fruits are first pulped, for the extraction of the pulp and juice, before the wine production. The must is then prepared, making some adjustments: the soluble solids content (TSS) is corrected to 20 Brix, adding sugarcane juice; 100 ppm of SO_2 as potassium metabisulfite and the pectinase enzyme (0.5%) are generally added, for degradation of the pectin enzyme and facilitation of yeast fermentation. Most of the fermentation is conducted using *S. cerevisiae* for 7–10 days at 22°C. After stamping and filtration, the wine is treated with bentonite and bottled with 100 ppm of SO_2 as potassium metabisulfite. A sweet fortified wine, known as “Dashehari,” is made by stopping the fermentation by adding 10% (v/v) mango brandy after 5 days of fermentation. For making sweet wine, sugar cane juice (5 g/L) is added to mango wine. The alcohol content of mango wines varies from 5% to 13% and wines usually contain low levels of tannins (Swami et al., 2014), ash of 0.27 g/100 g, soluble solids of 5°Brix, specific gravity at 30°C of 0.9812, and total acidity of 0.38% (as citric acid) (Chakraborty et al., 2014).

Obisanya et al. (1987) studied the fermentation of mango juice into wine using locally isolated *S. cerevisiae* and *Schizosaccharomyces* species of palm wine and they concluded that *Schizosaccharomyces* yeasts were suitable for the production of sweet mango wine and *Saccharomyces* yeasts were suitable for the production of dry mango wine with a higher ethanol level (Swami et al., 2014).

14.4.1.2 Jabuticaba Spirit and Wine

In Brazil, although most fruit production is destined for *in natura* consumption, beverage production is presented as a potential alternative for the use of fruits such as jabuticaba (*Plinia jabuticaba* Berg). It is a typical Brazilian fruit, belonging to the family Myrtaceae, which, although considered appropriate for both *in natura* consumption and for the industry, has limited trade due to its high perishability, which compromises the quality, mainly the external aspect. The fruits are black, the bark and pulp taste sweet and it has low acidity. Jabuticaba is used for the production of spirit, compote, jelly, jeripoga (sort of artificial wine, easier to prepare), vinegar, and wines.

The jabuticaba pulp contains most of the nutrients like carbohydrates, iron, calcium, phosphorus, vitamin C, and niacin. In the bark, there are good doses of pectin and anthocyanin which presents antioxidant function. The integral use of the fruit is the best option to take advantage of all its benefits, since the bark is the part of jabuticaba rich in phenolic compounds. The production of fermented beverages is an alternative to add value and increase the useful life of the product, in addition to being an extract with higher biological value, rich in phenolic compounds capable of controlling or preventing diseases (Gonçalves and de Souza, 2014).

The studies conducted by de Sá et al. (2014) have shown that, like wines, commercially available jaboticaba fermented beverages also have antioxidant potential, associated with vasorelaxant capacity, which may be an important ally for cardiovascular protection. Quercetin and ellagic acid are examples of the phenolic compounds identified in jabuticaba, where the vasodilator effect is well established (Khoo et al., 2010; Nishida and Satoh, 2013; Yilmaz and Usta, 2013; Li et al., 2012).

There is information on literature on the optimization of fermentation conditions and the use of jabuticaba for the production of spirits (Duarte et al., 2011). By adjusting the parameters Brix and temperature, it was possible to elaborate a distillate of jabuticaba with great sensorial acceptance, once fermentation conditions (e.g., temperature and °Brix) can exert both positive and negative influence on the quality of beverage.

14.4.1.3 Papaya Wine

Papaya (*Carica papaya*) is a tropical fruit widely grown throughout tropical and subtropical regions such as Australia, Hawaii, and South-East Asia. Papayas are high in protein, fat, fiber, and carbohydrates in comparison to other fruits. It is also a rich source of vitamin A, potassium, and carotenoids. Papaya has also been used for the production of papaya wine with alcohol content reported to be 11.3% (Chakraborty et al., 2014).

S. cerevisiae var ellipsoids is the yeast normally used in the production of all wines (Okoro, 2007). Some authors have showed that a very good table wine was produced from mature papaya pulp with *S. cerevisiae* isolated from a local brew beverage, *burukutu* (nonalcoholic beverage produced from sorghum (*Sorghum vulgare*)) (Umeh and Udemezue, 2015). In another study, Lee et al. (2013) investigated the effects of sequential inoculation of yeasts *Williopsis saturnus* var. *mrakii* NCYC2251 and *S. cerevisiae* var. *bayanus* R2 on the volatile profiles of papaya wine. Inoculation of *S. cerevisiae* after 7 days fermentation with *W. saturnus* produced papaya wine with more acetate esters and fruitiness than the control (simultaneous inoculation). The study showed that sequential inoculation of non-*Saccharomyces* and *Saccharomyces* yeasts at a certain inoculum ratio may be a valuable tool to manipulate yeast succession and to modulate the volatile profiles and organoleptic properties of papaya wine.

14.4.1.4 Raspberry Spirit and Wine

Raspberries (*Rubus idaeus* L.) present high polyphenolic phytochemicals, particularly flavonoids such as anthocyanin pigments, which give raspberries their characteristic color. The phytochemicals in raspberries may have a significant antioxidant activity and may act as a protectant against biological oxidative stress in mammalian cells (Weber and Liu, 2002). Phenolic acids, such as *p*-coumaric, caffeic, ferulic, and ellagic acids, are commonly found in raspberries (Häkkinen et al., 1999). Raspberry fruits that have no standards for *in natura* consumption is used in the production of juices, jam, and sweets; however, in some regions, for example, Campos do Jordão—Brazil, raspberry producers are looking for new alternatives for the use of small and crushed raspberry fruits (Duarte et al., 2010).

Duarte et al. (2010) performed a fermentation of the raspberry pulp using 16 yeast strains (*S. cerevisiae* and *S. bayanus*). After a screening step, strains CAT-1, UFLA FW 15 and *S. bayanus* CBS 1505 were previously selected based on their fermentation characteristics and profile of the metabolites, mainly volatile compounds. The characterization of the aromatic volatile compounds profile was in agreement with a sensorial analysis showing that the UFLA FW 15 presented the best results for the production of raspberry wine. The raspberry wine produced by this yeast was characterized in sensoria analysis by descriptors such as *raspberry*, *cherry*, *sweet*, and *strawberry*. Furthermore, based on the characteristics of the raspberry wine produced, the raspberry fruits showed good potential for use in the production of an alcoholic fermented beverage. The technology used has revealed an alternative use for small raspberry fruits, providing a new industrial use for this fruit (Duarte et al., 2010).

There is still little information about distillated raspberry beverages. In the study of González et al. (2011), the authors found that the total volatile compounds and anhydrous ethanol contents were 200.1 g/hL aa and 41.3%, respectively, in raspberry spirit. These concentrations were within the corresponding guidelines fixed by the European Council (Regulation 110/2008), since there is no legal restriction for fruit spirits. In addition, the mean concentrations of methanol in beverage (113.9 g/hL aa) were much lower than the maximum levels of acceptability fixed for red raspberry (1200 g/hL aa) distillates. These results showed that raspberry can be used as substrate to produce alcoholic beverages with high quality and safe for the consumers. As stated by Duarte et al. (2010) for raspberry wine, the spirit production is a viable alternative for the use of raspberry fruit.

14.4.1.5 Orange Wine

Orange is the most important citrus fruits cultivated throughout the tropical and subtropical areas of the world. Oranges are highly perishable products, susceptible to bacterial and fungal attacks. A number of steps are taken for storage and preservation. Among these measures are fruit cooling, extraction, juice concentration at about four or more times, and preparation of dry concentrate.

The quality of orange wines produced depends greatly on the yeast strains employed in the fermentation process. Okunowo et al. (2005) studied the efficiency of several native yeasts from various sources in the production and quality of orange wine. The *S. cerevisiae* var. *ellipsoideus* (isolated from yam) produced a moderate level of alcohol (5.61%) and appears to be the best microorganism for the production of orange wine. On the other hand, *S. carlsbergensis,* isolated from cane molasses, produced wines with higher alcohol content (6.80%).

Studying the volatile composition of Kozan orange wine, Selli et al. (2003) found 75 volatile components including terpenes, alcohols, esters, volatile phenols, acids, ketones, aldehydes, lactones, and C13-norisoprenoids. Among these, isoamyl alcohol, 2-phenylethanol, linalool, terpinene-4-ol, and ethyl-4-hydroxy butanoate were the main components contributing to the wine aroma along with ethyl hexanoate, ethyl octanoate, citronellol, and eugenol.

Santos et al. (2013) studying the use of orange juice do produce a citric spirit showed that the fermentation by yeast *S. cerevisiae* UFLA CA1174 was characterized by high kinetic parameter values, which were related to ethanol production. Furthermore, volatile compounds such as methanol and acetaldehyde, which negatively influence the quality of the beverage, were not found or were present at low levels. Sensory analysis showed good acceptance, despite tasters were not familiar with the orange-derived beverage. The authors conclude that orange fruit showed good potential for use in production of a distilled beverage.

In another report, Selli et al. (2002) studied the effect of bottle color, storage temperature, and storage time on the browning of orange wine. The results showed that the use of brown bottles and the short-time storage reduced the browning in orange wines; however, storage at two different temperatures did not significantly affect the browning index. Fernández-Pachõn et al. (2014) reported for the first time that melatonin in orange juice during alcoholic fermentation. It was observed that total melatonin underwent a marked sevenfold increase from day 0 (3.15 ng/mL) until maximal value at day 15 (21.9 ng/mL). Melatonin level in fermented orange juice (21.9 ng/mL) was higher than that found for fresh fruits. Results indicated fermentation of orange juice induces a gradual synthesis of melatonin with a rise from days 7 to 15 of alcoholic fermentation. Taking these results into account, enhancement in melatonin content of orange juice during fermentation could be due to both the presence of its precursor, tryptophan, and the new synthesis by yeast. When comparing melatonin amounts in fermented orange juice with the content in other fruits, it can be suggested that this beverage is a promising source of this bioactive compound, and the consumption of fermented orange juice could induce higher beneficial effects than orange juice alone.

Escudero-López et al. (2013) evaluated for the first time the influence of controlled alcoholic fermentation on bioactive compounds profile and the antioxidant capacity of orange juice. This process enhanced total flavanone and carotenoid content and antioxidant capacity of orange. The fermentation process of orange juice could be finalized at days 9–11 because in this time interval fermented orange juice presented the maximum values of total flavanones and antioxidant capacity and a significant increase of total carotenoids. In a second study, orange juice was subjected to alcoholic fermentation during 10 days and subsequent pasteurization to obtain a potential low alcoholic orange beverage. The changes in bioactive compounds and antioxidant capacity after thermal treatment were evaluated in order to establish the composition of this novel product which is important for its potential applications as functional food. The orange beverage showed lower flavanone and carotenoid content than orange juice but remained at comparable levels to other pasteurized orange juices. Thus, the orange beverage could be considered an excellent source of bioactive compounds with antioxidant capacity (Escudero-López et al., 2016).

14.4.1.6 Pineapple Wine

Pineapple (*Ananas comosus* L., Family-Bromeliaceae) is an important tropical fruit that is consumed in many parts of the world as fresh and processed product. In recent years, it has become one of the most demanded exotic fruits. It has a high nutritive value and a rich source of vitamins A, B, and C along with several minerals such as calcium, phosphorus, and iron (Chakraborty et al., 2014).

Dellacassa et al. (2017) studied a vinification method for pineapple juice that avoids dilutions of the fruit, which diminishes the quality and complexity of the final wine. Wines obtained with the conventional *S. cerevisiae* strain were analyzed by gas chromatography, and a total of 61 volatile constituents were detected in the volatile fraction and 18 as glycosidically bound aroma compounds. It was reported, for the first time in pineapple wines, the presence of significant concentrations of lactones, ketones, terpenes, norisoprenoids, and a variety of volatile phenols.

Okeke et al. (2015) produced a mixed fruit wine of pineapple and watermelon using *S. cerevisiae* isolated from palm wine. In general, the percentage alcohol produced from the fruits used for the fermentation by the yeast strain was above 2%. Some of the by-products include carbonyl compounds, alcohols, esters, acids, and acetals, all of them influencing the quality of the final product.

Chanprasartsuk et al. (2010) found that citric acid was the main acid in fresh pineapple juice and that its concentration decreased during fermentation, along with the generation of lactic acid, which resulted in the decrease of pH value in the fermented juices of these samples. This study investigated autochthonous yeasts and their functions in the spontaneous fermentation of freshly crushed pineapple juice samples. The other wine yeasts and, in particular, *Saccharomyces* yeasts, were not found in any of sampled fermentation systems that is apparently different from the other fruit juices. These findings suggested that the freshly crushed pineapple juice may possibly have some effects on the other autochthonous yeasts having important role in alcoholic fermentation. *H. uvarum* and *Meyerozyma guilliermondii* were the main yeast species observed in the fresh and fermented juice.

14.4.2 Challenges in the Use of Fruits for Beverages Production

Fruits wines are characterized by peculiar aromatic notes, antioxidant potential, phenolic composition, alcohol content, and other parameters. These wines could be prepared from nutritionally diverse, highly perishable, underutilized tropical, subtropical, or temperate fruits. This could help to reduce postharvest and production losses, increasing consumption, export, cultivation, and commercialization of fruits. Besides, it could also generate profits for growers and the existing wine industry. The progress achieved so far to improve the quality of fruit wines should help the commercialization of these wines, thus, collaborating with the wine industry economy. However, there is still a need for improvements in several aspects, among which we can mention selection of yeast strains for specific type of fruit; improvements in the processing of fruits, as in the extraction pulp and/or juice; improvements in post-fermentation stages, such as in the maturation and storage to avoid loss of quality (aromas, antioxidants, color, etc.).

Another point of concern is the need to act in changing the perception of wine consumers toward a greater acceptance of fruit wines, especially those of high-quality nutritional content with different aromas, flavors, and appearance in addition to the dominant commercial grape red wine.

14.5 Sweet Sorghum

Belonging to the family *Poaceae*, sweet sorghum (*Sorghum bicolor (L.) Moench*) was originated in East Africa and widespread in other regions such as South Africa, Europa, Australia, and the United States (Gnansounou et al., 2005). This plant stands out from other crops due to its high adaptability to adverse environmental conditions, such as drought resistance, high heat, and different soil types. Hence, it is adapted to subtropical and temperate regions, being specially indicated for regions with water restrictions. It is a crop with short a life cycle, varying between 120 and 130 days, presents low implementation costs, low water requirements, and seed propagation (Almodares and Hadi, 2009; Rezende and Richardson, 2017).

Besides having rapid growth, sweet sorghum is a multipurpose crop. Grains can be used as a food source; sugary juice extracted from its stalks can be applied in ethanol, syrup, or jaggery production; and bagasse and leaves may be used to animal feed or organic fertilizer (Datta Mazumdar et al., 2012). Sorghum is one of the main cereal crops consumed as a source of proteins and calories by poor population in many countries in Africa and Asia. Juice and grains extracted from it present a balanced nutritional profile containing proteins, essential amino acids, and minerals. Also, carbohydrates present in grains and steam can be used as a promising substrate to produce alcoholic fermented beverages (Barcelos et al., 2011; Baidab et al., 2016).

Sugar content in sweet sorghum juice varies from 12.5°Brix at the beginning of harvest up to 17°Brix during maturation. However, genetic improvement experiments in Brazil have already obtained genotypes that can reach up to 21°Brix (Purcino, 2011; Zabed et al., 2014). Its composition is mainly sucrose, glucose, and fructose, while sugars like maltose and raffinose are also present in lower concentrations. Sugar compositions usually found in this plant is 85% sucrose, 9% glucose, and 6% fructose, being able to present huge variations according to the environmental conditions (Gnansounou et al., 2005; Eggleston et al., 2013). The content of sugars makes sweet sorghum an interesting substrate for alcoholic fermentation. Below, we present a brief description of the current state of the art of sorghum use in the production of fermented and/or distilled alcoholic beverages.

14.5.1 Alcoholic Fermented Beverages From Sweet Sorghum

To be used as a substrate in the production of alcoholic fermented beverages, sweet sorghum must undergo through the malting process. Amylases present inside grains are activated, degrade the endosperm starch and release fermentable sugars. As well, proteases release free amino nitrogen (FAN), a nutrient essential to yeast growth during fermentation (Hassani et al., 2014; Rodríguez et al., 2017). Although sweet sorghum juice already has available sugars for fermentation and do not need malting, studies reporting its use to produce alcoholic beverages without distillation were not found.

Among the alcoholic fermented beverages produced from sorghum, beer is one of the most common. Sorghum grain has been traditionally used in Africa to produce beer for many years. The process usually includes malting, drying, milling, souring, boiling, mashing, and spontaneous alcoholic fermentation. However, some variations in the process may occur depending on geographic location (Lyumugabe et al., 2012). Among the traditional African beers, we can cite Pito (Djameh et al., 2015), Otika (Oriola et al., 2017), and Impeke (Claver et al., 2011). In general, to produce these beverages, the grains are submitted to malting process by adding water and incubating at room temperature. After 2 or 3 days, malted grains are dried under sun heat and grounded. Water is added to sorghum malt and the mixture is heated to activate enzymes and degrade starch. The fermentation is carried out spontaneously, resulting in beverages with low ethanol content and slightly acidified due to the presence of lactic bacteria

(Claver et al., 2011; Djameh et al., 2015; Vieira-Dalodé et al., 2015; Oriola et al., 2017).

The traditional African sweet sorghum beers are usually produced in household basis and small scale and consumed without downstream processing. There is no filtration to remove cells and debris from grains or pasteurization to reduce microbial load. Characteristics such as low ethanol concentration and high acidity can be justified due to the lack of control in inoculation. Microorganisms that do not produce much ethanol, like lactic bacteria, can consume available substrate without necessarily influence the alcohol content. Besides, the brewing process may not be sufficient to release enough fermentable sugars. However, even though there are some obstacles to be overcome, African beer plays an important in nutritional, economic, and cultural aspects of people's life.

14.5.1.1 Distilled Beverages From Sweet Sorghum

Both sorghum grains and juice are used to produce distilled spirits. The grain processing can be done using alternative technologies. Saccharification by solid-state fermentation is a technique well developed in China. Instead of malting and brewing the grains, they are cooked and inoculated with fungi to degrade starch (Zheng et al., 2012).

Distilled spirits from cereals have a huge impact on China economy, where it produces more than 5 million metric tons per year. Sorghum grains are used to produce distilled spirits like reported by Zheng et al. (2012) and Xing-Lin et al. (2017). Some processes use controlled inoculation, but mostly grains are cooked, diluted, and submitted to spontaneous fermentation.

In Brazil, the main manufacturer of distilled spirit from sugar cane, named "cachaça," there is a well-established market, but deeply dependent on sugar cane availability. In this context, sweet sorghum enters as an alternative or complementary substrate due to its life cycle and sugar composition. Sorghum harvesting is precisely during the off-season of sugar cane, a period when distilleries do not produce due to the lack of substrate. In this way, producers could have working distilleries during all year when complementing its production with sorghum.

Most studies about sorghum implementation approach its application in bioethanol production. However, a similar concept can be applied to alcoholic beverages. It has already been showed that distilled spirit produced from sweet sorghum is possible and results in a product with a considerable high concentration of aromatic compounds (Guimarães, 2013). We have recently used sweet sorghum for fermentation using *S. cerevisiae* to produce a distilled beverage. It was possible to note in this work that ethanol yield, aromatic composition and sensorial acceptance were very similar to the ones obtained with sugar cane juice. Besides aromatic and sensorial characteristic, another fact that supports the fermentation of sweet sorghum for production of distilled beverage is the possibility of using the same infrastructure used in the manufacture of cachaça.

14.5.1.2 Microbiota and Products Characteristics

The main production *bottleneck* faced when using sweet sorghum as a substrate for alcoholic fermentation is the availability of microorganisms well adapted as starter cultures.

Generally, distilled or non-distilled beverages produced from sweet sorghum have been produced using spontaneous fermentation. Spontaneous fermentation is carried out without control of the microbial community where usually, non-*Saccharomyces* yeasts are observed at the beginning of the process, and *S. cerevisiae* population develop as ethanol concentration increases. Beyond yeasts, lactic acid bacteria (LAB), molds, and some *Bacillus* spp. are also present. Alcoholic fermentations produced in this way are susceptible to risks like slow or stuck fermentations, increasing in contaminations and heterogeneity between batches (Rodríguez et al., 2010).

The *S. cerevisiae*, heterofermentative *Lactobacillus* and many others lactic bacteria are found in African sorghum beers fermentations (Lyumugabe et al., 2012). The presence of LAB in these beers, which has its growth favored by the presence of high sugar levels and free amino acids released during malting, justify the obtainment of slightly acid beverages (Hassani et al., 2014).

Challenges faced during spontaneous fermentations are overcome at the industry by inserting starter cultures in the process. As beverages' quality is directly related to the fermentative process, selected strains with better adaptability to the environment and higher production of aromatic compounds are essentials. The dominance of only one strain results in a homogeneous product, standardization between batches and fewer contaminations. However, variability in organoleptic characteristics decreases in this situation (Campos et al., 2010; Mukisa et al., 2012).

The insertion of mixed inoculations has been considered as a possibility to solve this problem. While *Saccharomyces* are superior producers of ethanol, non-*Saccharomyces* have been shown to be greater produces of aromatic compounds. Therefore, mixing both cultures in a controlled manner can bring both benefits (Avicor et al., 2015).

The secondary metabolites generated during the fermentative process are essential for sensory characteristics of fermented beverages. As already stated, different yeasts are able to produce distinct volatile compounds, such as esters, volatile fatty acids, and higher alcohols from sugars and amino acids. Ethyl acetate and ethyl lactate are the main compounds responsible for the typical flavor of Chinese spirits. Indeed, Chinese spirits produced from sorghum grains presented content of ethyl acetate and total esters higher than spirits produced from wheat, corn, barley, or rice. Also, the distilled spirit showed an increment on higher alcohol and decreasing on aldehydes concentrations, resulting in a product with soft-sweet taste (Xing-Lin et al., 2017).

14.5.2 Challenges and Innovations in the Use of Sweet Sorghum for Beverages Production

The search for special and functional beverages has stood out in the last years. Alcoholic beverages produced from sweet sorghum can be included in this scenery due to the presence of complex B vitamins, calories, antioxidants, amino acids, and minerals (Rodríguez et al., 2017).

Furthermore, there has been increased interest in sorghum as a gluten-free cereal to substitute gluten-rich cereals in the diet of people suffering from celiac disease. Germination and fermentation of sorghum grains have been shown to be viable and to improve nutrition value. Opening the opportunity for development of fermented beverages, especially gluten-free beers, to assist this market (Elkhalifa et al., 2005; Elkhalifa and Bernhardt, 2010).

Among some challenges to use sweet sorghum juice, there are the harvest seasonality and a large amount of biomass that needs to be transported and stored. Grains are easier to store when compared to stalks because of sweet sorghum's juice composition. Hence, it is decisive for distilleries to process the stalks as fast as possible to avoid loss of productivity (Regassa and Wortmann, 2014).

On the other hand, when analyzing the use of sorghum grains, the main difficulty is the malting process. The sorghum beer supply is still insufficient in the global market due to the low quality of its malt. Some of the criteria evaluated for malt quality are α-amylase and β-amylase activity, responsible for converting the starch in soluble sugars, and free amino acids essentials for yeast growth. Germination time has been proved to have a huge impact on these characters, followed by germination temperature and degree of steeping, and further studies need to be done to find better processing conditions (Hassani et al., 2014; Djameh et al., 2015).

Although tannins present in sweet sorghum grains supply protection against insects, birds, and weather damage, in high concentrations its presence inactivate extracted malt amylases. However, when in concentrations up to 2%, they affected microbial metabolism during the fermentative process of Chinese distilled spirit. There was an increase in flavor compounds, such as esters, ethyl acetate, including ethyl caprylate, and higher alcohols (Taylor et al., 2006; Xing-Lin et al., 2017). One possibility to overcome and take advantage of both situations would be the selection of crops with suitable tannin levels to produce beverages.

14.6 Conclusions

Considering the above-mentioned examples of alcoholic beverages produced from differentiated substrates and their characteristic, it is possible to note the existing potential and how much has yet to be explored in this context. Besides generating added value beverages, the use of the cited substrates such as fruits, coffee by-products, and cheese whey makes it possible to meet the market demand for new products, in addition to reducing losses in some cases. For the full development of these new beverages, several aspects still need to be improved, as reported above, especially those related to some up and down stream stages and to the use of microorganisms suitable, specifically, for each substrate.

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15

TRADITIONAL FERMENTED BEVERAGES IN MEXICO

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15.1 Introduction

Throughout the year civilizations had to evolve their way of life for survival, going from a nomadic life involving hunting of animals and fruits gathering for food to transform to perform in a sedentary civilization, in a specific territory dedicated to animal husbandry and agriculture (Howey and Frederick, 2016).

This generates new challenges such as the storage or reserve of raw materials for future uses to respond to a shortage due to environmental, cultural variability, etc. In the particular case of food storage is more complicated by the perishable nature that characterizes them. An important and fundamental tool that has been used since the beginning of civilizations is the fermentation of food to produce new products, as well as their preservation (Marsh et al., 2014). This ancestral technology can be defined as the process where microorganisms or metabolites have been used to produce desirable biochemical changes (Blandino et al., 2003; Quintero-Salazar et al., 2012). The first records date from 6000 AC in the Middle East that is very likely to happen by accident.

The fermentation process in food was artisan and without any knowledge about microorganisms' key role, which can be native therefore the microflora present in the substrate, in the environment or added with knowledge (Battistini et al., 2017). The first processed foods were bread, cheese, wine, and then extended to many others, this process in addition to serving as conservation improved the flavor,

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aroma, texture, nutritional value if as other attractive properties of these (Narzary et al., 2016).

Fermented foods are produced around the world using various manufacturing techniques, raw materials, and microorganisms, it is estimated that there are around 3500 foods worldwide (Misihairabgwi and Cheikhyoussef, 2017). In Mexico there are more than 200 fermented products. Although there are different genera and species of microorganisms that are used for production, there are only four main processes of fermentation: alcoholic, lactic acid, acetic acid, and alkaline fermentation (Valderrama et al., 2018; De Roos and De Vuyst, 2018).

Fermented beverages are other products of high relevance in civilizations, it is believed that the first fermented food was the grape (*Vitis vinifera*) to produce wine, as this fruit is easily contaminated, and the conditions and characteristics make this process optimal (Hugenholtz, 2013). Fermented drinks have played an important role in ancient cultures as they are related to food, culture, religion, politics, and economics (Bostan and Mahmooda, 2010; Murakami, 2016). The production processes have been passed from generation to generation, which has preserved as natural and cultural wealth.

Fermented beverages are widely accepted worldwide since they represent an important part of the products produced and consumed in the world. Their great attractions are related to the varieties of flavor that are produced, thanks to the varied concentrations of organoleptic factors that contribute to a large extent to the volatile compounds (Hugenholtz, 2013). Variations can occur between species, in the maturity of the raw materials (fruits, stems or grains, raw or cooked) used, and in the metabolism of the microorganisms (yeast and bacteria) used for the manufacture of the specific beverage (Ruiz and Rodarte, 2003).

Mexico is a country that has a wide and varied cultural richness, due to various factors such as: its location, its extreme reliefs, contrasting ecosystems that represent a wide source of raw materials such as a wide range of fruits, cereals, roots, and cacti grains (Ibarrola-Rivas and Granados-Ramírez, 2017). This has helped for centuries the diversification of a wide range of products as well as the quality in them, a proof of this is the production of food and beverages considered worldwide as a world heritage site. There is an extensive list of fermented traditional beverages which are distributed by the different and so varied reliefs, climates, and ecosystems of the national territory (Fig. 15.1), depending on the production of particular raw material of the regions as well as the resources of each one. In this chapter, 10 of the most popular fermented drinks produced and consumed in Mexico are discussed: tequila, mezcal, sotol, bacanora, pulque, tejuino, colonche, pozol, tepache, and tuba presenting the elaboration process, denomination, its origin and distribution, as well as particularities of each of them.

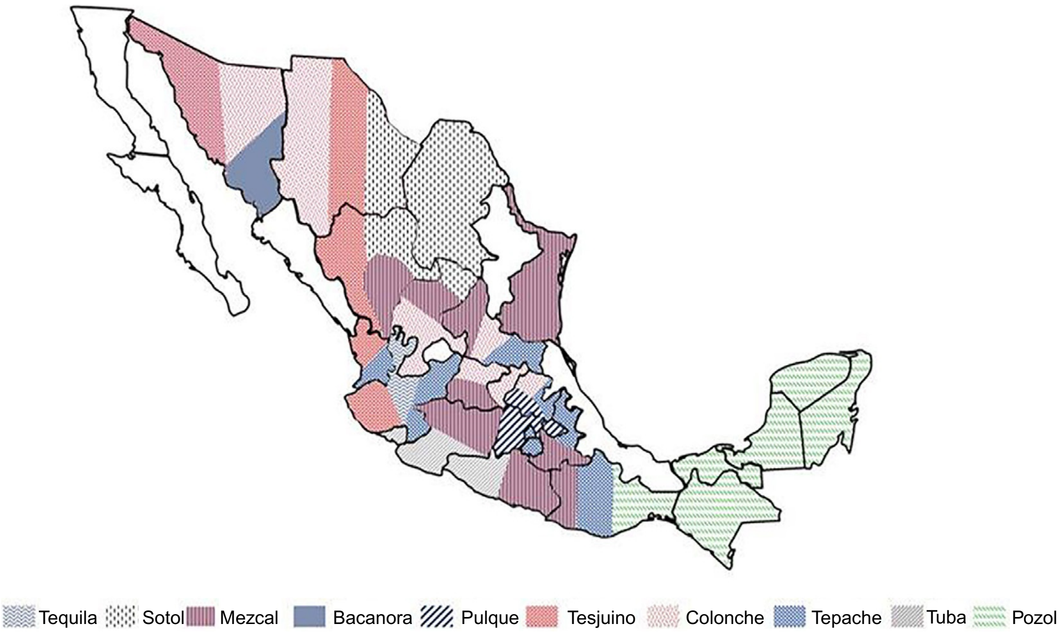

Fig. 15.1 Geographical distribution of the production of traditional fermented beverages in Mexico.

15.2 Fermented Beverages

15.2.1 Generalities

Beverages are essential for the population since it is a primary need so they have constant contact with them, these can be defined as all those liquids that the human being ingests including water, the main objective is to quench thirst and in some cases provide nutrients or calories in Mexico approximately 21% of the total energy consumed by adolescents and adults comes from beverages (Rivera et al., 2008; Valderrama et al., 2018). The beverage industry is a branch that is in constant development and innovation to meet the needs of the modern population, however there is a lot of history and traditions behind this industry. The drinks can be classified into two large groups. Nonalcoholic and alcoholic beverages.

15.2.2 Nonalcoholic Beverages

These as its name indicates are free of alcohol among which stand out: milk, coffee, juices and juices of fruits and vegetables, and carbonated beverages that are loaded with carbon dioxide to give effervescence to the liquid as sodas and mineralized waters, also tea and infusions, the latter being the most consumed beverage throughout the terrestrial globe only after water. Water forms the basis of all beverages, which contain other compounds such as CO_2, flavors, and sugars (Pelvan and Özilgen, 2017). The purposes of this chapter is not to delve into this type of beverages.

15.2.3 Alcoholic Beverages

Alcoholic beverages are understood to contain to some extent ethyl alcohol, which have been consumed since ancient times (Blandino et al., 2003). They are comprised of different types of beverages, of which they are classified as fermented beverages and distilled fermented beverages that differ by their method of elaboration, level of graduation that they reach, and their appearance.

Fermented beverages are produced as a result of an anaerobic process in which the sugar is hydrolyzed forming two products: ethyl alcohol and carbonic anhydride. This biotechnological process is produced owing to the intervention of microorganisms usually yeasts such as *Saccharomyces* that process carbohydrates of the raw material for consumption and survival that the ethanol produced is in fact a secondary product, a metabolic by-product that even the microorganism does not need or search for and all this with the help of its entire range of metabolites such as the enzymes produced, which help concretize the transformation process (Blandino et al., 2003). These are produced as result of fermentation of different substrates including fruits such as grapes, apples, citrus, among others, cereals such as barley, corn, wheat, rice, etc.

15.2.3.1 Distilled Beverages

These beverages have a base, thanks to the ferment that is exposed to a distillation process. This is a process where it is sought to concentrate on the alcohol obtained from the biotechnological process through evaporation due to heating and then to condensation as a result of cooling that manages to eliminate water and some other compounds by concentrating on the degree of alcohol between 15% and 45%. The drinks with these characteristics are whiskeys, rum, brandy, gin, liqueurs, and a group called brandy where we can find drinks like tequila and mezcal (Mosedale and Puech, 1998).

15.3 Traditional Fermented Beverages in Mexico

15.3.1 Distilled Fermented Beverages

15.3.1.1 Tequila

Tequila is a Mexican product obtained from the fermentation of sugars and distillation of agave juice (*Agave tequilana* Weber blue variety) (Nava-Cruz et al., 2015; Villanueva-Rodríguez et al., 2016), its production and commercialization are verified and certified by the Mexican Tequila Regulatory Council (CRT) (Alemán-Nava et al., 2017; Nava-Cruz et al., 2015). According to official Mexican standards (NOM-006-SCFI-2005), tequila is defined as: "Regional alcoholic beverage distilled from must, directly and originally prepared from the material extracted in the factory premises of an Authorized Manufacturer which shall be located within the territory specified in the Declaration for the Protection of Appellation of Origin Tequila (also called the Denomination of Origin Tequila—DOT)" (Mexican Secretariat of Economy, 2006). In this way, DOT means that tequila can be produced only in certain areas. Similar term is used in the European Union "protected designation of origin." This includes 181 counties belonging to five Mexican states, such as, 125 from Jalisco, 8 from Nayarit, 7 from Guanajuato, 30 from Michoacan, and 11 from Tamaulipas. With tequila designation of origin (DO), the product counts with international protection around the world with DOT registrations in countries like Canada, Costa Rica, Peru, European Union, China, among others (CRT, 2016).

Tequila is prepared from the heads of the Agave tequila Weber blue variety; the production begins when agave plants are ready to be cut for beverages production, that is, when they are 8–10 years old. Inflorescence is removed to concentrate sugars into the stem, and then the head is extracted from the ground (in México the head is also known as "piña"). Then, heads are transported to distillery in which a sample is analyzed to determine the reducing sugar content along with many other parameters to facilitate cooking (Pinal et al., 1997). The next phase is the cooking, where several biochemical reactions are involved. This step hydrolyzes the complex carbohydrates into simple sugars because these cannot be solubilized in water and they are not fermented, after this sugar extraction is made and stem residues are separated. When fermentation ends, and must have reached an alcoholic content of 5%–7%, distillation is undertaken in copper or stainless tanks or in columns. This process is conducted in two ways: where the first one is known as destroying; when the vinazas are eliminated from water ("heads" and "lines"), and the product obtained is named ordinary tequila with 25–30°GL. The second distillation is termed rectification and has the objective to concentrate ethanol and make separate the heads and lines, having at the end a 45–50°GL. The final content must be from 35 to 45°GL (NOM-006-SCFI-2005). Depending on the process after distillation, the liquor is named White tequila, youth or old gold, rested, and old extra (Campos-Montiel et al., 2013).

Depending on the percentage of sugars coming from agave used in the production of tequila, it is categorized in two ways: the first one is agave is 100%, in which fermentation is not susceptible to be enriched with other sugars compared to those obtained from tequilana Weber blue variety and its denominated as "100% de agave," "100% puro de agave," and "100% agave." The second category is Tequila, the product in which must is susceptible to be enriched and mixed jointly prior to fermentation with other sugars until a proportion not higher than 49% of the total reducing sugars expressed in mass units is reached. This maximum enrichment up to 49% of total reducing sugars expressed in mass units cannot be performed with sugars coming from any other agave variety. In all, 51% of the total reducer sugars can be increased with sugars extracted from the Agave tequilana Weber blue variety (CRT, 2016). Also, based on the characteristics of the process subsequent to distillation, tequila is classified as silver, young or gold, aged, extra aged, and ultra-aged (CRT, 2016).

The Secretariat of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA, for its acronym in Spanish) reported that the annual export of this product reaches about 184.4 million liters to more than 740 international destinations, making these traditional Mexican drinks the most representative of our country. Reported sales were 182.9 million liters in 2015, and an increase (between January and August 2016) in exports of 133.7 million liters. This represents an annual growth of 4.7 in the volume of tequila sold abroad. The top 25 export destinations for tequila are: United States, Germany, Spain, France, Japan, South Africa, United Kingdom, Colombia, Brazil, Canada, Panama, Latvia, Singapore, Greece, Australia, Italy, Turkey, Chile, United Arab Emirates, China, Russia, Belgium, the Philippines, South Korea, and the Netherlands. According to statistics reported by the CRT, it is mentioned that together these 25 nations represent exports for about 129.3 million liters of this drink (SAGARPA, 2016).

For the preparation of tequila, from January to August 2016, 632.2 thousand tons of agave were used, which means an increase of 18.1%, during the year 788.9 thousand tons of agave were used for 228.5 million tequila liters production in the country. With this, it is pointed out that Mexico has 181 municipalities with DOT in five states, which protects this national beverage and makes exclusive its elaboration in the country and was the first product to obtain this distinction at the national level (SAGARPA, 2016).

15.3.1.2 Mezcal

Mezcal is a name that comes from the nahuatl mexcalli "baked agave," provided to traditional distilled alcoholic drinks produced in many rural areas of Mexico, from some northern states up to south states. These liquors are obtained from the fermented sugars of the cooked stems of species of the genus *Agave*, commonly named "maguey" (Aguierre-Dugua and Eguiarte, 2013).

In mezcal production are used from more than 14 maguey species and the main used species are *Agave salmiana*, *Agave angustifolia*, *Agave cupreata*, *Agave esperrimia Jacobi*, *Agave webericela*, *Agave durangensis*, and *Agave potatorum* (Aguierre-Dugua and Eguiarte, 2013; Hinojosa, 2010; Mendoza, 2007; Villanueva-Rodrigues and Escalona-Buendia, 2012). Actually Mexico produces annually 365 thousand tons of agave (Nava and Martínez, 2014).

Mezcal is an alcoholic beverage with a different odor and taste, colorless, or slightly amber when it is rested or aged in oak wood, considered a traditional drink consumed widely on both mundane and ritual occasions. According to what is established in NOM-070-SCFI-1994, it is considered mezcal, the regional alcoholic beverage obtained by distillation and rectification of musts prepared directly and originally with sugars extracted from mature agave heads. One particular, and very valuable characteristic of the mezcal industry is that it has production exclusivity, called the DO. As the DO is a distinctive sign referring to a geographic region, this product cannot be appropriated individually or privately, which means that it is an element of National Heritage (Avilés et al., 2017; de Leon-Rodrígues et al., 2008; Nava and Martínez, 2014).

At the national level, mezcal has denomination of origin for nine states of the Mexican Republic: Durango, Guerrero, Guanajuato, Michoacán, Oaxaca, Puebla, San Luis Potosí, Tamaulipas, and Zacatecas. Each of these regions has endemic maguey, which makes the mezcals have different characteristics like flavor, color, texture, and smell. The protected name of the mezcal is linked to the area of its manufacture: Mezcal from Oaxaca, Mezcal from Guerrero, etc. (Villanueva-Rodrigues and Escalona-Buendia, 2012).

Oaxaca is the “home of mezcal” and the main reference in Mexico that coalesces the image and promotion of mezcal and its importance lies in being the main producer of mezcal (66%), in addition has the best participation in the following indicators: maguey plants (36.8 million), number of maguey producers (13,231), mezcal factories (590), and volume of mezcal packaged for export (49%) (Avilés et al., 2017).

One of the main characteristics of mezcal is its artisanal production process, which has been transmitted from parents to children. For its elaboration are cultivated magueyes or wild, and other raw materials, it is presumed that during the process of fermentation and distillation neither chemicals are added, or other substitutes are used, which guarantees the production and consumption of a natural mezcal and that in agreement with the NOM. 070 it has the category of 100% agave. The general process for mezcal production is integrated in various steps: harvesting and cutting agave, cooking, mashing, or milling to obtain agave juice rich in sugar, fermentation, first distillation, second distillation, cask maturation (if the mezcal is rested or aged), and finally the bottling. In contrast to tequila, mezcal fermentation is carried out using the whole mash from the agave heart, including the fibers (Hinojosa, 2010; Kirchmayr et al., 2017; Martínez-Gándara, 2008).

The difference between mezcals, mainly in the flavor, comes from the different agave species used, which have many variety of terpenes compounds, the fact that the agave leaves can be used in mezcal fermentation, the variations in the ripening stage of agave, the cooking of agave that can be in ground holes with burning wood and heated stones that produces furans and smoky volatiles and are retained in the agave, and some herbs or other natural materials (e.g., worms) can be added to enrich the volatile profile of mezcal (Villanueva-Rodrigues and Escalona-Buendia, 2012).

The main products in the mezcal fermentation process are: ethanol, methanol, *n*-propanol, 2-butanol, and acetic acid (Hinojosa, 2010; Villanueva-Rodrigues and Escalona-Buendia, 2012). The main by-product generated in the mezcal production is the agave bagasse as a solid residue and vinasses, from which 15 kg of bagasse, on wet basis, and between 8 and 15 L of vinasses are generated by every liter of mezcal produced. In Mexico, 141 tons of bagasse and about 8 million liters of vinasses monthly are produced. The solid residue can be used as source of energy in mezcal factories, where most of the time mezcal production is the only viable economic activity. In mezcal production, vinasses is possible and also the recovery of many polyphenol compounds, some organic acids (lactic and acetic acid), melanoidins and inorganic species such as sulfates and phosphates salts (Hinojosa, 2010; Robles-González et al., 2012; Salazar et al., 2016).

The mezcal marketing, whose incursion into international markets is relatively recent, in 2015 reached a marketing volume of 1.9 million liters to destinations such as the United States, Germany, Spain and the United Kingdom, with sales estimated at 16.7 millions of dollars (SAGARPA, 2017).

15.3.1.3 Sotol

Sotol is an alcoholic beverage, the processes to obtain this beverage is by distillation of fermented musts of heads or pineapples (De la Garza-Toledo et al., 2008) from plants of the genus *Desylirion* native from Chihuahua, Mexico desert and has been used as a food or medicine source for more than 7000 years. This plant from the *Nolinaceae* family is succulent, polycarpic, and perennial, sotol leaves are thin, stiff, and narrow, the plant core is known as “head or pineapple” and has a weight from 20 to 40 kg in mature plants (Cruz-Requena et al., 2013; Pardo-Rueda et al., 2015). The plant can survive under cold winter and extreme summer and lives up to 150 years (Cruz-Requena et al., 2013). At present, they are the raw material for the production of the *Sotol* (Melgoza Castillo and Sierra Tristán, 2003). The plants grows in regions of the Mexican desert, the Mexican Institute of Industrial Property granted the protection of the denomination of origin to the states of Chihuahua, Durango y Coahuila, located to the north of Mexico (Lachenmeier et al., 2006; Melgoza Castillo and Sierra Tristán, 2003). Nowadays, about 16 species have been identified, and the state of Coahuila six or seven species have been recognized (Cruz-Requena et al., 2013).

Sotol is constituted by carbohydrates such as fructose, glucose, sucrose, and fructooligosaccharides, and fructosyl polymers. Also, sotol stores inulin-type fructans as the major carbohydrate. The fructans in sotol have been categorized as graminans and branched neo-fructans and its prebiotic effect has been demonstrated (Leach and Soboli, 2010). Due this important feature of these carbohydrates, different extraction methods of water-soluble carbohydrates from sotol have been evaluated (Pardo-Rueda et al., 2015), the extracts of sotol (*Dasylirion leiophyllum*) under pre-enzymatic treatment at 40°C have been reported, and the presence of inulin, sucrose, glucose, and fructose sugars have been demonstrated.

There are some studies about the characterization of the Sotol (De la Garza-Toledo et al., 2008) studied the sugar content of raw material of *Dasylirion cedrosanum*, the plant was collected from different areas and different years seasons. The authors reported that the total sugar content (g/L) of sotol pineapples is 24.54–37.92, in function of the location where the plants were collected. On the other hand, De la Garza et al. (2010) studied the chemical profile of six commercial sotols by solid-phase microextraction-gas chromatography. The authors reported that the principal compound detected was ethanol in concentrations of 769.7–1713.75 mg/mL and methanol of 102.376–296.32 mg/mL. Another compounds such as ethyl acetate, propanol, 1-butanol, and 3-hydroxy butanol were detected in the analysis of six commercial sotols. Also, Cruz-Requena et al. (2013) characterized chemical and molecular properties of the sotol plants (male and female) and the authors reported that no significant differences between pines of male and female plants were found in the chemical characterization, and no difference in alcohol percentage values were detected between fermented male and female plants.

Of all recognized species of *Dasylirion*, only two species have features and properties to be used in the industry; *D. cedrosanum* and *Dasylirion durangensis* (De la Garza-Toledo et al., 2008).

The global market of sotol is limited because of its chemical composition is scarce and confused (De la Garza et al., 2010); however, the liquor demand has increased at domestic and international level because of the consumption and production (Cruz-Requena et al., 2013). The study of characterization of plant, sotol beverage, and responsible microorganisms in the fermentation process have been carried out.

15.3.1.4 Bacanora

Bacanora is a regional alcoholic beverage from the state of Sonora, its name being derived from the town of Bacanora in the eastern part of the state, this distillate is elaborated mainly in the mountain regions of Sonora (Bahre and Bradbury, 1980; Millán-Soto et al., 2016). This spirituous beverage can be considered as variety of mescal; etymologically, the word mescal, or mescal, derived from the Nahuatl metl, maguey and izcalli, boiled, meaning boiled maguey (Mapes and Basurto, 2016a,b).

According to the NOM-168-SCFI-2004 "Bacanora 100% Agave," is a product obtained from the distillation and rectification of must, prepared directly and originally with the sugars extracted from the grinding of mature heads of *Agave angustifolia* Haw., hydrolyzed by cooking, and subjected to fermentation alcoholic with yeast. Bacanora can be white, rested, or aged (Álvarez-Ainza et al., 2015) (Ley de fomento para la producción, industrialización y comercialización del bacanora del estado de Sonora).

Denomination of origin was considered "illegal" for a period of 77 years. It did not have the permission or the support of the state or national government for its production, nevertheless, clandestine production continued a small scale, maintaining the tradition and taste of the beverage. It was until in 1992 they were issued a series of norms to be able to develop the industry again.

With a letter filed on May 28, 1999, at the Federal Delegation of the Secretariat of Commerce and Industrial Development, located in Hermosillo, Sonora, and received by the Mexican Industrial Property Institute on June 30, 1999, the Government of State of Sonora, applied to the Mexican Institute of Industrial Property for the General Declaration of Protection of the Bacanora Denomination of Origin (General declaratory of protection to the designation of bacanora origin 2016).

In October 2000, the Institute agreed to issue a declaration of protection to the "Bacanora" denomination of origin, which is confined to 35 municipalities in the east and in the state of Sonora and was published a month later in the Official Gazette of the Federation (NOM-168-SCFI-2004; Salazar, 2007).

In the small vinatas of the sierra, the bacanora is produced in an artisanal way. Precisely, what distinguishes the bacanora from other mezcales is the procedure followed in the second distillation of the liquid, commonly known as rectification, but in Sonora it is called "resaque." During the resaque, the person in charge of supervising the elaboration of the bacanora—called "vinatero" in Sonora, the first distillate is collected in a container, which comes out of the alembic: they are the heads, which has very high alcohol content. As soon as one notices that, in the flowing liquid, a group of small superficial bubbles that take time to break, the so-called pearls-form, the container is changed. Finally, when he perceives that the bubbles disappear quickly, he changes the container again, because the last thing that is obtained from the distillation—the so-called tails—is composed almost entirely of water. The last step is to try the bacanora and mix it with the heads to adjust its alcohol content, or, as the vinatero say, to "compose" it. It will be with the palate how, in the last instance, the flavor and the graduation of the bacanora will be defined which, in general, is between 40 and 50 degrees (Núñez, 2001; Gutiérrez-Coronado et al., 2007).

Bacanora steps of production described by Salazar and Mungaray (2009) are presented in the following:

(A) Agave collection/selection. Plants that reach at least 8 years of age are selected.

(B) Jimado. Once the agave plants mature after several years of growth, they are harvested by removing the pointed blades with a manual cutting instrument (coa), until leaving the plant core, which is called pineapple or maguey head.

(C) Cooking or Tatema of the "pineapples" (heart of the agave). Once the "pineapples" were fried, they are placed in a natural oven, which consists of a circular excavation forming a large container covered with stone and mud. Previously, rocks with firewood were heated until they reach "bright red" color. The cooking process is completed in 2–4 days. The purpose of this process is to convert the starches and carbohydrates by hydrolysis into digested sugars for the yeast in the fermentation process.

(D) Grinding. The cooked "pineapples" are pulverized or cut into pieces to facilitate the extraction of the juice or handmade by blows. The product is placed in containers with water to start its fermentation.

(E) Fermentation. It is the process by which the sugars contained in the must are converted into ethyl alcohol. For this, the presence of yeasts, microscopic fungi that are found naturally in the "pineapples" sewn of mezcal, is used. The ground maguey pulp is placed in barrels or casks with water, which are covered immediately after the weather and left to ferment for 4–5 days.

(F) Distillation and condensation. It is the process by heating the fermented agave musts so that their more volatile components go to steam. These are returned to a liquid state by condensation and by cooling, homogeneous and high-quality alcohol is naturally obtained.

(G) Standardization. It is the process by which an experienced vintner combines the product obtained in the different stages of the distillation to determine the quality of the bacanora.

15.3.2 Non-Distilled Fermented Beverages

15.3.2.1 Pulque

Pulque, *metoctli*, *iztac octli*, or *poliuhqui octli* as it was known by the aztecs, is one of the oldest traditional non-distilled alcoholic fermented beverages in Mexico. It is believed that this beverage has their origin in Mesoamerica. The hypothesized is that the pulque was used as a dietary supplement and risk-buffering food in ancient Teotihuacan (150 B.C. to A.D. 650) (Correa-Ascencio et al., 2014). The pulque was consumed in religious ceremonies, for its nutritional, health-promoting properties, and alcoholic content (Valadez-Blanco et al., 2012). It is a beverage white, viscous, slightly acidic, and with an alcoholic content between 4 and 7°GL (Escalante et al., 2016).

The pulque is produced by fermentation of sap (aguamiel) obtained from the plant Maguey, mainly from the species *Agave atrovirens* and *Agave americana*; however for its production other species can also be used as *Agave ferox*, *Agave mapisaga*, and *Agave salmiana* (Torres-Maravilla et al., 2016). The fermentation process is spontaneous traditionally carried on by the natural flora present in the sap (indigenous microorganisms), even if it is possible using a previously fermented pulque as a starter.

The production of pulque takes place in four general stages: (1) maguey castration, (2) scraping and aguamiel extraction, (3) seed preparation, and (4) pulque fermentation. In *maguey castration*, the embryonic floral peduncle that surrounds the floral bud in the mature plant (5–10 years of growth) of maguey is destroyed. The central leaves of the plant are eliminated to leave a hole in the center of the plant. After this process, the plant is left in a maturation process for 3 months. In *scraping and aguamiel extraction*, the cavity surface is scrapped to promote the production of sap. The sap (aguamiel) flows and accumulates in the cavity. The aguamiel accumulated is extracted twice per day. This liquid is stored in plastic containers. In *seed preparation*, the inoculum is prepared for fermentation, in general, this is a portion of previously produced pulque. In this stage, 2 L of fermented pulque are added to 20 L of fresh aguamiel, it is incubated at room temperature for 1–4 weeks until the desired acidity and alcohol characteristics are obtained. And finally, in *pulque fermentation*, the fresh aguamiel is filtred and transferred to any container for their fermentation. The aguamiel is fermented during 12–24 h in wood, clay, or more commonly, in plastic containers (Escalante et al., 2016).

Exits different parameters to determine the quality and degree of fermentation in the pulque among which are the viscosity, due to exopolysaccharide synthesis by the microorganism, the acidity, and alcohol content. These parameters are related with the kind of fermentation that takes place during the process: viscous, acid, and alcoholic fermentation. The process is static and it is realized under non-aseptic conditions, therefore the beverage has a big population of microorganisms (Giles-Gómez et al., 2016). These microorganisms are a mixture of bacteria and yeasts such as *Zymomonas mobilis*, *Lactobacillus* spp., *Saccharomyces cerevisiae*, *Leuconostoc mesenteroides*, *Leuconostoc kimchii*, *Candida lusitaneae*, *Kluyveromyces marxianus*, *Microbacterium arborescens*, *Flavobacterium johnsoniae*, *Acetobacter pomorium*, *Gluconobacter oxydans*, and *Hafnia alvei* (González-Vázquez et al., 2015).

No standardized process exists for pulque production because producers do not control even basic variables such as temperature, inoculum, pH, Brix, among others. In artisanal production, the fermentation process changes due to geographic ubication, weather conditions, aguamiel quality, etc. Likewise, the final fermentation time is decided by the person skilled in pulque, but there are no defined parameters for this (Gómez-Aldapa et al., 2012). In spite of this, currently there are two Mexican standards related to the production of pulque. These are the NMX-V-022.1972-AGUAMIEL-HIDROMEL and the NMX-V-037-1972-PULQUE MANEJADO A GRANEL. The NMX-V-022.1972 refers to the quality of the mead for pulque production, while the NMX-V-037-1972 defines the alcohol content and the permitted pH limits that pulque must have for direct consumption (Escalante et al., 2016).

During the production of pulque many products are obtained that may be of interest in other industries. These include: honey of maguey, inulin, industrial alcohol, vinegar, gums, sour drinks, syrups, fruit, and fiber juices known as Ixtle (SAGARPA, 2015).

In the early to mid of the 20th century, pulque represented a potential economic resource in central Mexico, and it is still consumed in the region, although there is not much information about its microbiological stability (Gómez-Aldapa et al., 2011). It is important to mention that the main pulque producer is the state of Hidalgo, which in 2010 produced more than 206 million liters of pulque, equivalent to 82% of the total national production, followed by Tlaxcala with 13.3% and the State of Mexico with 2.58% (SAGARPA, 2015, 2017).

The consumption of pulque at the national level is low, mainly it is consumed in the states of Guadalajara, State of Mexico, and Puebla. Currently, there are different export plans to send pulque to different countries in America, Europe, and Asia. However, at the moment only agave with 21% alcohol products are exported to the United States (179,630 L/year) and Spain (8397 L/year) (Breña Cervantes et al., 2010). Therefore, being able to export to other countries represents a viable alternative to encourage pulque production at the national level.

15.3.2.2 Tejuino

Tejuino (Tesgüino, Teshuino, pozolli) is a traditional alcoholic beverage from northern and northwestern Mexico elaborated with nixtamalized corn mass fermented, so it contains probiotics. For this reason it is considering a functional food (Lacerda Ramos and Schwan Freitas, 2017).

Some authors called the fermented maize beer from northern Mexico (Applbaum and Levi, 2003), they evaluated the ethnographic contexts and found that the Tejuino is sacred drink among Rarámuri native Chihuahua Mexico people and their consumption reveals the organic social bonds in relations of production and exchange. Other native Mexican people in Mexico who consume tejuino are the yaquis, pimas, tepehuanos, and huicholes (Elizondo, 2016). Despite this beverage have been since the pre-Columbian civilization, it is still part of the actual Mexican culture. One drink of tejuino of 750 mL contains 12 g of alcohol (Panduro et al., 2017). Nutritionally the tejuino contains 74% of moisture, 2% of protein, 0.2% of crude fiber, 2.5 mg of iron, 0.03 mg of thiamine, 0.03 mg of riboflavin, and 0.29 mg of niacin, besides ethanol, lactic, and acetic acid (Wacher-Rodarte, 1995).

Tejuino is derived from “tecuin” that mean heart beating (Elizondo, 2016). Tejuino is a cold bittersweet beverage very refreshing that is served with lemon juice, a pinch of salt and shaved ice shell or lemon sorbet. The most of trade of tejuino is informal because it does not have an establishment, their distribution is through the “tejuineros,” people who adapted their bicycles to carry the containers and ice to prepare the drinks (Elizondo, 2016).

The first probiotic isolated from tejuino from Chihuahua, Mexico was *Bacillus megaterium* in 1974 (Ulloa et al., 1974). Recently, probiotic bacterial strains such as *Weissella cibaria*, *Leuconostoc citreum*, *Bacillus safensis*, *Pseudomonas brenneri*, *Pseudomonas psychrophila*, *Chryseobacterium bovis*, *Acetobacter tropicalis*, *Brochothrix thermosphacta*, *Corynebacterium calluane*, *Kurthia gibsonii*, and *Pantoea vagans*, have been isolated from tejuino from Jalisco, Mexico (Silva et al., 2017). Yeast as *Candida guilliermondii*, *Hansenula anomala*, *Saccharomyces kluyveri*, *Saccharomyces cerevisiae*, has been isolated from Chihuahua, Durango, Jalisco, Nayarit, Oaxaca, Sinaloa, and Sonora, Mexico (Romero-Luna et al., 2017).

The probiotics have the capacity to produce a wide range of prebiotics compounds, thus *Pantoea anthophila* strains isolated from tejuino were studied as β-galactosidase producers and were found that probiotics synthesized several galactooligosaccharides (GOS), mainly the disaccharides such as allolactose [Gal-β(1→6)-Glc] and 6-galactobiose [Gal-β(1→6)-Gal]; and the trisaccharides as 3′-galactosyl-lactose [Gal-β(1→3)-Gal-β(1→4)-Glc], 6-galactriose [Gal-β(1→6)-Gal-β(1→6)-Gal], 3′-galactosyl-allolactose [Gal-β(1→3)-Gal-β(1→6)-Glc], and 6′-galactosyl-lactose [Gal-β(1→6)-Gal-β(1→4)-Glc] (Yañez-Ñeco et al., 2017).

Its production has been inherited from generation to generation in families as part of the culinary art (Elizondo, 2016) and vary among the ethnic groups. The process of the elaboration is artisanal, the processing time and the amount of ingredients can be different, but the hydration of nixtamalized corn mass, sugar addition, cooking-boiling, and fermentation are four essential steps for its production (Fig. 15.2). In Mexico brown sugar cane or unrefined brown sugar are the principal sweeteners used, and these have an important metabolic role for the fermentation process and sensory properties. The race of corn is another important factor for the sensory properties of tejuino and has reported the use of Apachito and Blue native corns for its production (Suárez et al., 2013).

The sugar is metabolized by tejuino biota by three pathways: Embden Meyerhof Parnas, hexose monophosphate, and Entner Doudoroff. During fermentation, the tejuino biota have an important function in the development of the sensory properties. So, *Saccharomyces cerevisiae* is the principal alcohol producer, this yeast

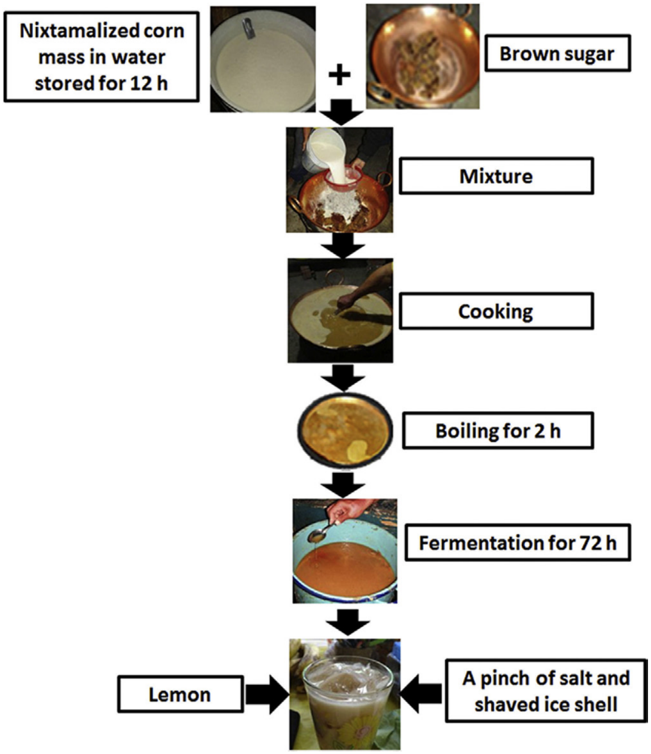
Nixtamalized corn mass in water stored for 12 h
+
Brown sugar
Mixture
Cooking
Boiling for 2 h
Fermentation for 72 h
Lemon
A pinch of salt and shaved ice shell

Fig. 15.2 Flow diagram used in the artisan production of the Mexican corn fermented beverage (tejuino).

is maintained on the surface of the utensils. Homolactic and heterolactic bacteria produce organic acids as lactic and acetic which confer to tejuino its distinctively refreshing and sour flavor. The other yeasts generate organic compounds that contribute to turbidity, aroma, flavor, and viscosity (Sangwan et al., 2014). Actually, there does not exist information about the by-products from tejuino processing, prices, and international market.

15.3.2.3 Colonche

It is prepared by spontaneous fermentation of the juice of prickly pears (tuna) of several cactus species, particularly *Opuntia streptacantha* Lem. (cardon tuna), *O. orbiculata* Salm-Dyck (tuna pintadera), and *O. leucotricha* D.C. (Duraznillo). It is a traditional drink that consumes indigenous people from Chihuahua and Sonora, especially the Tarahumaras and Yaquis, as well as the mestizos of Zacatecas and San Luis Potosí, Querétaro, Guanajuato, and Hidalgo, but only in the season of the year when the nopales produce fruits (Mapes and Basurto, 2016a,b).

The aztecs called this drink "nochoctli" (from Nahuatl, "nochtli," tuna and "octli," wine). While the word "colonche" comes from Nahuatl "coloa" which means stoop, because of its alcohol intake effect (Mapes and Basurto, 2016a,b).

The colonche is defined as a pleasant-tasting alcoholic beverage resulting from the fermentation of red tunas (Hamdi, 1997). It has a sweet flavor due to the presence of unfermented sugars (7%–9% of sugars), with a certain degree of viscosity and low alcohol content (4%–5%), but which over time acquires a sour taste (Díaz, 2001).

Colonche is made from the pulp of the prickly pears and the juice is obtained by crushing the fruits, the juice is boiled, it is left to cool and it is added into an old or tibico colonche (constituted by dextran's and yeasts which develop in the nopal stalks and are used as starter cultures) to begin fermentation which is monitored trying to avoid an overproduction of organic acids which can alter the flavor and other characteristics of colonche making it less pleasant to drink (Herrera, 2007; Mapes and Basurto, 2016a,b).

No process has been developed for the preservation of this drink that undergoes bacterial alterations quickly (Díaz, 2001). Therefore, it must be consumed at the exact point of fermentation before its flavor is undesirable. There is lack of scientific and technological information on the processing of the colonche and on the microbial flora that participates in the respective biotechnological processes. It is based on the hypothesis that it is a mixed natural fermentation where both yeasts and various harmful bacteria intervene, often causing unpleasant odors and tastes (Díaz, 2001).

Several yeast strains have been isolated from colonche fermentation such as *Saccharomyces cerevisiae*, *Torulopsis*, *Candida valida, and Candida colliculosa*, being *Saccharomyces cerevisiae* the most found described in this product (Ulloa and Herrera, 1990; Bravo, 1991). Lactic acid bacteria (LAB) have been found to intervene during fermentation, but the strains have not been identified yet.

León (2010) made a comparative fermentation to produce colonche, one fermentation was performed spontaneously, and the present microflora was isolated, while other fermentation was conducted with controlled parameters (prickle pear juice was pasteurized, *Saccharomyces cerevisiae* was inoculated, and the temperature was controlled). The result of the spontaneous fermentation was an alcoholic strength of 5.8 degrees, total soluble solids of 4.20°Brix, citric acid production, pH 3.92, and *Aspergillus niger* and *Saccharomyces cerevisiae* strains were isolated. As the result of the controlled fermentation the net percentage of alcoholic degree was 6% alcoholic strength, with the following physical characteristics: red color, bittersweet taste and alcohol, and pleasant smell of sugar and alcohol, resulting a drink is suitable for human consumption from *O. streptacantha*, concluding that if the fermentation is performed under controlled conditions, high-quality product can be obtained, standardized to commercialize this antique Mexican beverage.

15.3.2.4 Tepache

Tepache is a mildly alcoholic non-distilled-beverage with prehispanic roots, traditionally consumed and elaborated in Mexico. *Tepache* is consumed principally in entities as Mexico city, but diverse variants are prepared and consumed along Mexican country as: Hidalgo, Puebla, Morelos, San Luis Potosí, Oaxaca, Jalisco, and Nayarit, although their processes varies, in ancient times was prepared with maize, it is made from fruits such as pineapple (*Ananas* spp.), apple (*Malus communis*), and orange (*Citrus aurantium*) (Bamforth, 2014; Herrera Suárez, 2003; Mapes and Basurto, 2016a,b) and has also been reported their production from other raw materials as *jobo* (*Spondias mombin*) or *timbiriche* (*Bromelia karatas*) (Godoy et al., 2003).

Tepache is prepared through traditional methods from pineapple peels and flesh, brown sugar, water, and spices, this kind of artisanal fermentation occurs in wood barrels, at room temperature during 1–4 days, contrarily to distilled beverages. Alcohol is not desirable like main product, but in *tepache* a low ethanol concentration (<1%) is generated, with a partial sugar consumption providing a sweet taste, with production of lactic, acetic, and other volatile compounds enhancing their organoleptic characters, although if fermentation is prolonged a sour and disgusting flavor is generated due to acetic acid (Corona-Gonzalez et al., 2013; Moreno Terrazas Casildo, 2005).

It has been demonstrated that this kind of fermented beverage is a rich source of microorganism due to the spontaneous and noncontrolled nature of the process, but some microorganisms has been identified as *Bacillus megaterium*, *Bacillus subtilis*, and yeast such as *Saccharomyces cerevisiae*, *Pichia membranifaciens*, *Candida boidinii*, and *Candida inconspicuous* (Mapes and Basurto, 2016a,b). *Tepache* is a drink that can relive environmental problems through the reutilization of pineapple shell produced by canning industries for their transformation in a beverage for human consumption, with nutrients and vitamins from fermenting microorganisms (Romero-Luna et al., 2017) and has also reported the presence of bacteria from genera *Lactobacillus* with potential in bacteriocin production, making the beverage as remarkable source of antimicrobial compounds (de la Fuente-Salcido et al., 2015), and also the presence of strains as *Leuconostoc mesenteroides* with potential in bacteriocin and dextran production, finally a extracellular polymer with wide properties including prebiotic activities (Sarwat et al., 2008).

15.3.2.5 Tuba

The tuba is a drink that is obtained by the fermentation of the sap of different palms, in Mexico mainly from *Cocos nucifera*. This beverage, also called "palm wine," and its preparation was introduced by the Spaniards to Mexico from the Philippines, along with the palm tree in the 16th century. The term Tuba is a word of Philippine origin, whose name designates the liquid that emanates from the sap of the palm (Velazquez-Monreal et al., 2011). On the other hand, the natural constituents of the sap include macronutrients, micronutrients, and phytochemicals (so-called "phytonutrients") namely, sucrose and other polysaccharides, vitamins, minerals, amino acids, organic acids, phytohormones, and phenolic compounds (Yuan et al., 2013).

The coconut tree *Cocos nucifera* is distributed in tropical and subtropical regions of Africa, the Caribbean, and South America. Its greatest variability occurs in Southeast Asia and secondly in the Caribbean. In Mexico there are two contrasting types of coconut, in terms of genotypic characteristics, "niu kafa" (wild type) and "niu vai" (domesticated type), distributed all over the coasts of the Atlantic and the Pacific oceans, respectively, both introduced independently by the Spanish in the 16th century (Granados and López, 2002; Manivannan et al., 2018). *Cocos nucifera* L. is widely distributed in islands and tropical coastal areas around the world, between 26°C north and south latitudes; also, can be found at heights of up to 1200 masl. It is a tropical plant that thrives best in climates without marked seasonal fluctuations, with an average temperature higher than 20°C, average annual rainfall of 1000–1800 mm, being able to withstand greater rainfall in well-drained soils (Granados and López, 2002). The name "tuba" corresponds to the sap extracted from plants. Each palm tree, in its mature stage, can provide 1–2 L per day of fresh sap and tuba is sold in a price between 1.8 and 2.7 euros per liter, depending on establishment, distributor, or its presentation (Rosado, 2002).

The newly obtained sap fermentation product is brown, but action of the microorganisms clarifies it, producing a white color. It maintains its color and its distinctive flavor until 2 h after being harvested. After about 5 h the fermentation process begins. The tuba is commonly consumed served with nuts, or mixed with fruits, water or sugar (called "tuba compuesta," composed tuba) (Gutiérrez Solís, 2016; Rosado, 2002). If the tuba is left undisturbed for 8 days, it becomes a tasty vinegar very useful in the local cuisine, and excellent for the pickling of vegetables used in the famous bread soup, served at weddings and baptisms. Freshly fermented is a refreshing drink, and after fermentation is used to make vinegar or brandy. It is rich in sugars, vitamin C, phosphorus, amino acids, and minerals; in addition, it is auxiliary against the gastritis and the parasites. The fermentation process is quick and begins immediately after the sap collection; 2 h later, the produced wine is aromatic, soft, and sweet and may contain a 4% of alcohol (Velazquez-Monreal et al., 2011). The fermentation process is started by yeast (principally *Saccharomyces cerevisiae*) (Stringini et al., 2009).

Besides in the Philippine-original tuba *Saccharomyces chevalieri* is the principal microorganisms responsible of fermentation (Kozaki, 1979), Mexican tuba contains 158 cultivable bacterial strains including LAB, mesophilic aerobic bacteria and yeast including *Lactobacillus lactis* and *Enterococcus faecium* (Romero-Luna et al., 2017) that have been identified as bacteriocin-producing strains (de la Fuente-Salcido et al., 2015) responsible for beverage production and related to its flavor and aroma, besides conferring indirect probiotic properties to the product (de la Fuente-Salcido et al., 2015).

Due to its rapid fermentation process, the fresh produced tuba is commercialized daily; if there is some product that cannot be sold, a thermal process must be performed to stop fermentation in order to avoid vinegar formation. In the same way, sap collected at noon hours is commercialized on the next day so it needs to be thermal processed too (Velazquez-Monreal et al., 2011).

In a commercial way, the Mexican tuba is a poorly known product because of its consumption principally by local costumers in relatively less settlements. Nevertheless, tourists can be of good opportunity in publishing this beverage due to the relationship between its production in tropical places and the constant touristic traffic in those places (Rosado, 2002).

15.3.3 Nonalcoholic Fermented Beverages

15.3.3.1 Pozol

In Mexico maize (*Zea mays* ssp. *may*) is one of the most produced and consumed crops, with economic, social, and cultural importance (Sweeney et al., 2013), their vast diversity allows their use in a large variety of foodstuffs (Mapes and Basurto, 2016a, b), as fermented beverages like *Pozol*.

Pozol is a nonalcoholic beverage, traditionally consumed in southeast Mexico, trough Maya region, especially in Chiapas, Tabasco, Campeche, Yucatán, Quintana Roo, and Oaxaca; by ethnic and mestizo populations (Mapes and Basurto, 2016a,b; Ulloa et al., 1987; Wachner, 2014), firstly for its preparation, maize grains are exposed to multistep process, called nixtamalization, which consists in cooking the grain in a calcium oxide solution (0.5%–2% w/w), after it is cooled in the water being cooked, washed to remove the pericarp and excess of alkaline solution for subsequent grounding (Villada et al., 2017), from this, a dough is formed into balls and wrapped in banana leaves for their posterior spontaneously fermentation at ambient temperature for 2–7 or even more days; the resulting dough is suspended in water and consumed as a refreshing drink, or in other cases used traditionally for relive conditions as diarrhea and fever (Cárdenas et al., 2014; Méndez-Albores et al., 2004; Wachner, 2014), with diverse variants in their consume, adding grounded cacao grains, sugar, honey, coconut pulp, or a set of dry, toasted, and milled chili (Wachner, 2014).

In this class of fermented foods has been identified a wide variety of microorganism coexisting during fermentative process as LAB, amylolytic lactic acid bacteria (ALAB), other class of bacteria, yeast, and filamentous fungi (Ben Omar and Ampe, 2000), but the mechanism through the fermentation performs remains unclear (Cárdenas et al., 2014), due to uncontrolled manner of preparation; however several species are always present where usually LAB represent between 90% and 97% of microflora, where genera as *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Weissella* predominates (Castillo-Morales et al., 2005), and other important issue in this fermented beverage is that their low fermentable sugar content in non-fermented dough that varies from 0.1 to 0.7 g of sugar per 100 g of nixtamalized dough due to the nixtamalization process, and therefore, starch in this fermentation comes crucial, where ALAB plays a key role; studies indicates at early stages, 40% of this class of bacteria are present, where strain as *Streptococcus bovis*, in particular, predominates and is presumed that this bacteria provides glucose and low-molecular weight maltooligosaccharides to non-amylolytic flora during the first stage in fermentative process (Díaz-Ruiz et al., 2003), other studies make remarkable facts that generation of other carbon sources such as xylooligosaccharides (XOS) during nixtamalization process can be exploited for non-amylolytic bacteria such as *Weissella confusa* isolated from *Pozol* samples (López-Hernández et al., 2017).

It has been reported that this class of artisanal fermentation can produce not only desirable organoleptic changes, but can also promote health benefits after consuming them, acting as prebiotic and probiotic strains sources; López-Hernández et al. (2017) recently proposed genera such as *Weissella* ssp., as potential probiotic, and in situ XOS production potential acting as prebiotics, or the isolate of bacteria with potential in prebiotics productions such as *Leuconostoc citreum* (Olivares Illana et al., 2002). Some studies present the *Pozol* and other Mexican maize products has the potential as protective in collateral effects of high sucrose intake in rats (Muñoz Cano et al., 2013), and other studies have reported that the regulation in lipid profile effect in maize LAB fermentation products (Banjoko et al., 2012), although the isolation of bacterial strains with antimicrobial substances production in solid fermentation providing protection against pathogens such as *Escherichia coli* (Ray et al., 2000), and enhancing nutritional characteristics as nitrogen content compared to non-fermentable dough (Haard, 1999).

15.4 Commercial and Market Opportunities

Approximately there may be around 5000 varieties of common and uncommon fermented foods and alcoholic beverages in the world (Tamang et al., 2017). In Mexico, there are around 200 fermented foods and beverages, but only a few have been investigated (Romero-Luna et al., 2017), this has generated that only a few drinks are produced in an industrialized way. However, it is important to emphasize that traditional Mexican drinks are more than just tequila, this without underestimating the great commercial contribution that tequila has won in the world, there is currently great potential to commercially exploit many beverages that are currently only produced by hand in some regions of the country.

Mexico is a country that traditionally consumes alcoholic beverages and this market is very important in the country's economy, the per capita consumption of alcoholic beverages in Mexico will accelerate by an average of 2.6% between 2014 and 2019 (Loreto, 2017). In the world, the Mexican alcohol market is in the seventh position and its production volume is constantly growing (Ramirez, 2017), so in the next few years, Mexico will be able to occupy the sixth position of world production.

Among the trends of consumption of alcoholic beverages of the population, domestic products are of special interest; these are craft wines, craft beer highlighting mainly agave alcoholic beverages such as tequila and mezcal. Although previously, mezcal was considered a cheap alternative to tequila and aimed at low-income groups, the modernization of production techniques has produced a very high-quality mezcal, successfully marketed among a new base of local consumers (Loreto, 2017), this aspect opens a door for the future market of nontraditional alcoholic beverages that are produced in an artisanal way. However, the potential for national commercialization and future exports will depend on the fulfillment of hundreds of factors demanded by the market, for example: the standardization of the technologies used in the fermentation, the fermentation techniques used by the ethnic communities show that they have been trapping the indigenous microflora to obtain a spontaneous and energy-efficient technique for bioconversion in useful edible products (Narzary et al., 2016).

Currently, there is no control of microorganisms involved in the process, so there may be a risk to the health of consumers, in this sense, researchers and investors should make efforts for the introduction of technologies that imply the improvement of quality, the improvement of the process, and the improvement of the raw material, the production of hygienic and pure microbial starter cultures (Narzary et al., 2016). Traditional Mexican drinks are also a source of probiotics (Romero-Luna et al., 2017), so that fermented beverages can have beneficial effects on health, an intensive collaborative research work is needed, for the standardization and commercialization of these fermented products due to their flavor and high nutritional value.

It is important to highlight that the commercial potential of Mexican alcoholic beverages will directly benefit rural populations where raw materials are traditionally produced, many of these people have low incomes, which would also have a significant contribution to the poverty reduction. This would contribute to the sustainability of the regional economy by promoting the livelihood of the rural population.

Another challenge associated with the industrialization and commercialization of fermented beverages in Mexico is the natural availability of the substrate, for example, the agave, which is the raw material used in many fermented beverages, takes 7 years to reach maturity (Escalante et al., 2016). Also, the standardization of stable processes for obtaining fermented products is a necessity, in the artisanal production of pulque since the honey water collection is done daily and not in a single process (as in the case of tequila or mezcal). This generates many uncontrollable factors such as the beginning of fermentation and the microorganisms that participate in it. Despite this, several companies developed by pulque producers from the states of Puebla, Tlaxcala, and Hidalgo export pulque to Europe, Central America, and the United States. The United States is the main pulque market, mainly in cities where there are large settlements of Mexican immigrants (Jaurez Rosas, 2015). However, the studies that were carried out showed that the industrial processes implemented to standardize production (pasteurization, the addition of preservatives, antioxidants, and texturizing agents) also affected the sensory and functional characteristics traditionally presented by an antral drink (Escalante et al., 2012), so this must be a point of consideration in future commercial and industrialization initiatives.

There is no doubt about the great cultural contribution that Mexico has given the world and that makes Mexican cuisine declared by UNESCO as a world heritage, and alcoholic beverages are part of that gastronomic tradition. In Mexico ancestral communities use fermented foods and beverages for their stimulating, functional and nutritional characteristics, a way to rescue this cultural heritage is to industrialize the production of beverages (Romero-Luna et al., 2017), to improve traditional technologies and offer greater added value while rescuing cultural heritage. Different types of microorganisms that also produce nutraceutical compounds can be isolated (Narzary et al., 2016). To guarantee the commercial success of the Mexican alcoholic drinks, a new scientific investigation must be carried out to demonstrate the main functional properties of beverages and microorganisms, modifying the traditional production to ensure a quality product, without the beverages losing their autochthonous characteristics (Escalante et al., 2016).

15.5 Perspectives

Mexico is a country with a wide diversity of flora and fauna. Its natural wealth has been exploited in the different industries such as cosmetic, pharmaceutical, and food. However, the alcoholic beverage industry is another area with potential application. The Tequila, Sotol, Pulque, Mezcal, among others, are a traditional alcoholic beverage, their production is based on the fermentation process.

There are some reports about the characterization of the microbial community present in the fermentation of these beverages realized the characterization of the bacterial present during the fermentation of pulque, and the authors reported that the principal microorganisms presents during the process were of genus *Lactobacillus*, *Lactococcus*, *Zymomonas*, *Erwinia*, etc. On the other hand, the characterization of volatile compounds of the alcoholic beverage has been studied (De la Garza et al., 2010). However, the detailed study of the microbiota present during the fermentation process can be carried out to evaluate its possible potential in the production of compounds with biotechnological application. Also, the compounds produced by microorganisms present in fermentation, could be added to the beverage and give to it an extra value. Likewise, the carbohydrates present in the raw material of the beverage, such as glucose, sucrose, fructooligosaccharides, etc. could be used in the food industry.

For thousand years microorganism has been used for improving the nutritional, sensory, and safety properties in food, including beverages. In beverage fields, Mexico has a broad diversity, despite the most worldwide popular beverages. Their diversity also includes a set of traditional beverages not widely known, generally result from empirical knowledge and transmitted from generation to generation, some has been carried out through, noncontrolled process depending on environmental conditions, as humidity, temperature, and also the availability of multiple raw materials (Lacerda Ramos and Schwan Freitas, 2017). Such represent a source of interest compounds and health benefits, contributing to develop new products and expanding knowledge in food science and in a few cases food industry residue exploitation, improving with nutritional and functional properties from traditional and millenary beverages, although it is necessary to most extensive investigation for promoting and understanding their benefits, microbial interactions, and production process (Romero-Luna et al., 2017).

15.6 Final Remarks

In the history of Mexico, from pre-Hispanic times to the present, alcoholic beverages have been a cultural reference and a bastion of national gastronomy. The drinks used by our ancestors have a great potential to generate development and wealth to the new populations through industrial exploitation. To continue with the research directed to the characterization of the microorganisms associated with the production of traditional fermented beverages in Mexico is recommended, this is to improve the initial cultivation and develop a high-quality product. The use of starter cultures would guarantee hygienic processing. This information would be very useful in the design of innovative fermentation equipment which also contributes to improving working conditions and profits for rural populations. New alternatives for the use of by-products and waste generated in fermentation must also be generated; for this, biorefinery tools can be used. The new investigations should also be oriented to the characterization of the probiotic potential of ancestral drinks and of all the microorganisms that naturally participate in the fermentation.

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Further Reading

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THE SCIENCE OF BEVERAGES
VOLUME 5

FERMENTED BEVERAGES

Edited by Alexandru Mihai Grumezescu and Alina Maria Holban

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